Supplementarily file 1

Designing of multi-epitope vaccine against Varicella zoster virus (VZV) using immunoinformatics and structural analysis: In silico study

Mohamed J. Saadh[1], Mareb Hamed Ahmed[2*], Rafid Jihad Albadr[3], Gaurav Sanghvi[4], R. Roopashree[5], Aditya Kashyap[6], A. Sabarivani[7], Zafar Aminov[8], Waam Mohammed Taher[9], Mariem Alwan[10], Mahmod Jasem Jawad[11], Ali M. Ali Al-Nuaimi[12]

[1]Faculty of Pharmacy, Middle East University, Amman, 11831, Jordan
[2]College of Dentistry, Alnoor University, Mosul, Iraq
[3]Ahl al Bayt University, Karbala, Iraq
[4]Marwadi University Research Center, Department of Microbiology, Faculty of Science, Marwadi University, Rajkot-360003, Gujarat, India
[5]Department of Chemistry and Biochemistry, School of Sciences, JAIN (Deemed to be University), Bangalore, Karnataka, India
[6]Centre for Research Impact & Outcome, Chitkara University Institute of Engineering and Technology, Chitkara University, Rajpura, 140401, Punjab, India
[7]Department of Biomedical, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India
[8]Department of Public Health and Healthcare management, Samarkand State Medical University, Samarkand, Uzbekistan
[9]College of Nursing, National University of Science and Technology, Dhi Qar, Iraq
[10]Pharmacy College, Al-Farahidi University, Baghdad, Iraq
[11]Department of Pharmacy, Al-Zahrawi University College, Karbala, Iraq
[12]Gilgamesh Ahliya University, Baghdad, Iraq

Figure S1. The tertiary structure of vaccine that predicted by Galaxyweb. A: Before refinement B: After refinement**.**

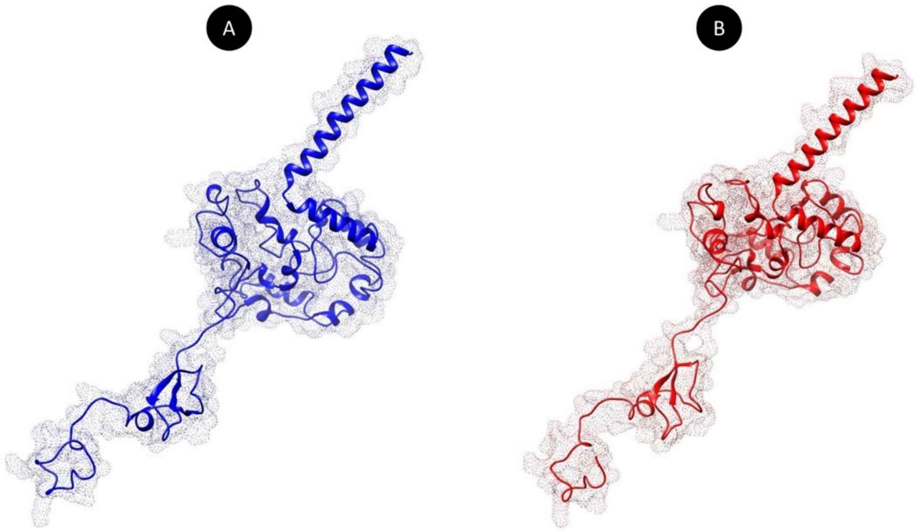

Figure S2. Disulfide engineering map. The yellow light showed the potential disulfide bond.

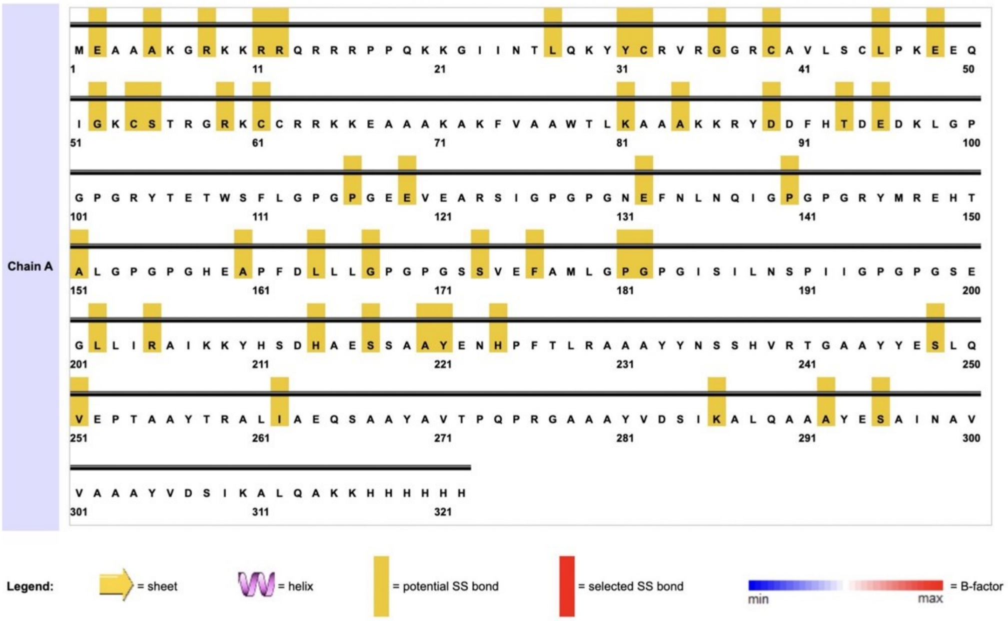

Table S1. The conformational B-cell epitopes.

Number	Residues	Number of residues	Score
1	A:H318, A:H319, A:H321, A:H322	4	0.967
2	A:E2, A:A3, A:A4, A:A5, A:K6, A:G7, A:R8, A:K9, A:R11, A:R12	10	0.954
3	A:A292, A:E294, A:S295, A:N298, A:A299, A:V300, A:V301, A:A302, A:A303, A:A304, A:Y305, A:V306, A:D307, A:S308, A:I309, A:K310, A:A311, A:L312, A:Q313, A:A314, A:K315, A:K316, A:H317	23	0.793
4	A:M1, A:R14, A:R15, A:R16, A:P17, A:P18, A:Q19, A:K20, A:K21, A:G22, A:I23, A:I24, A:N25, A:T26, A:L27, A:Q28, A:K29, A:Y30, A:Y31, A:C32, A:R33, A:V34, A:R35, A:G36, A:G37, A:R38, A:C39, A:A40, A:V41, A:L42, A:S43, A:C44, A:L45, A:Q50, A:I51, A:G52, A:K53, A:C54, A:S55, A:T56, A:G58, A:R59, A:K60, A:C61	44	0.744
5	A:V120, A:E121, A:A122, A:R123, A:S124, A:I125, A:G126, A:P127, A:G128, A:P129, A:G130, A:N131, A:E132, A:F133, A:N134, A:L135, A:N136, A:Q137, A:I138, A:G139, A:P140, A:G141, A:P142, A:G143, A:R144, A:M146, A:R147, A:T150, A:A151, A:L152, A:H214, A:A215, A:E216, A:Y234, A:N235, A:S236, A:S237, A:H238, A:V239, A:R240, A:T241, A:G242, A:A243, A:A244, A:Y246, A:E247, A:S248, A:L249, A:Q250, A:V251, A:E252, A:P253, A:T254, A:Y257, A:A268, A:Y269, A:A270, A:V271, A:T272, A:P273, A:Q274, A:P275, A:R276	63	0.644
6	A:D96, A:K97, A:L98, A:G99, A:P100, A:G101, A:P102, A:R104, A:Y105, A:T106, A:E107, A:T108, A:W109, A:S110, A:F111, A:S190, A:P191, A:I192, A:I193, A:G194, A:P195, A:G196, A:P197, A:G198, A:E200	25	0.555

Table S2. Molecular docking between represented MHC alleles and epitopes.

MHC I Alleles	PDB ID	Peptide	Docking score	Delta G binding (kcal/mol)
H-2-Kd	2FWO	RYDDFHTDEDKL	-169.62	-8.8
H-2-Kk	1ZT7	HEAPFDLLL	-181.05	-9
H-2-Kb	1LK2	SSVEFAML	-178.55	-8.4

MHC II Alleles	PDB ID	Peptide	Docking score	Delta G binding (kcal/mol)
H2-IAb	1MUJ	AVTPQPRGA	-150.65	-26.1
H2-IAd	7RDV	YESLQVEPT	-152.65	-25.3
H2-IAb	1MUJ	VDSIKALQA	-100.56	-24.7

Table S3. Comparative Evaluation of Different Linkers in Multi-Epitope Vaccine Design.

Parameter	GPGPG Linker	AAY Linker	GGGS Linker	EAAAK Linker	Validation Method
Flexibility (RMSF, Å)	1.2 ± 0.3	1.4 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	MD Simulations (100 ns)
Epitope Exposure (SASA, %)	85 ± 3	82 ± 4	78 ± 5	70 ± 6	Solvent Accessible Surface Area Analysis
Antigenicity (VaxiJen Score)	0.52 ± 0.03	0.51 ± 0.04	0.45 ± 0.05	0.38 ± 0.06	VaxiJen v2.0
TLR2 Docking Score (kcal/mol)	-280.8 ± 12.3	-275.6 ± 11.7	-265.2 ± 14.1	-255.9 ± 15.8	PatchDock / MM-GBSA
Immune Response (IgG titer)	1:12,800	1:11,200	1:9,600	1:8,400	C-ImmSim (30-day simulation)
Structural Stability (RMSD, nm)	0.15 ± 0.02	0.17 ± 0.03	0.22 ± 0.04	0.25 ± 0.05	MD Simulations
Disulfide Bond Potential	High (3 predicted)	Moderate (2 predicted)	Low (1 predicted)	None	DbD2 Server

Table S4. MMGBSA result.

Energy component	Average Value during 100ns (TLR2-vaccine)
VDW	-153.81
ELE	-907.61
GB	1007.18
SA	-20.83
Total	-75.07

Table S5. Population coverage.

Population/Area	**Class Combined Coverage[a] (%)**	**Average-Hit[b]**	**pc90[c]**
Central Africa	78.74	9.91	4.23
Central America	2.19	0.20	0.92
East Africa	77.37	9.96	3.98
East Asia	64.49	6.79	2.53
Europe	100.00	36.55	29.97
North Africa	52.04	5.35	1.88
North America	100.00	37.09	30.45
Northeast Asia	78.53	9.16	4.19
Oceania	88.77	12.51	8.01
South Africa	62.45	6.71	2.40
South America	82.04	10.28	5.01
South Asia	91.25	11.88	9.21
Southeast Asia	46.10	4.32	1.67
Southwest Asia	40.63	4.03	1.52
West Africa	72.96	9.26	3.33
West Indies	64.55	7.01	2.54
World	**100.00**	**33.99**	**27.48**
Average	**70.71**	**12.65**	**8.20**
Standard Deviation	**24.64**	**11.17**	**10.01**

[a] projected population coverage

[b] average number of epitope hits / HLA combinations recognized by the population

[c] minimum number of epitope hits / HLA combinations recognized by 90% of the population

Table S6. Predicted MHC Class I and II Epitopes with Population Coverage.

Epitope Type	**Epitope Sequence**	**Population Coverage (%)**
MHC I	RYDDFHTDEDKL	70.65
	RYTETWSFL	89.90
	HEAPFDLLL	78.43
	SSVEFAML	56.80
	EEVEARSI	99.80
	NEFNLNQI	97.00
	ISILNSPII	87.76
	SEGLLIRAI	65.43
	RYMREHTAL	95.32
MHC II	AVTPQPRGA	79.56
	YHSDHAESS	98.34

	ENHPFTLRA	86.54
	YNSSHVRTG	99.32
	YESLQVEPT	98.30
	YSRPLISIV	76.67
	ESAINAVVA	78.22
	VDSIKALQA	74.54
	TRALIAEQS	75.67