Supporting Information for:

Molecular Mechanism of Be^{2+}-Ion Binding to HLA-DP2: Tetrahedral Coordination, Conformational Changes and Multi-ion Binding

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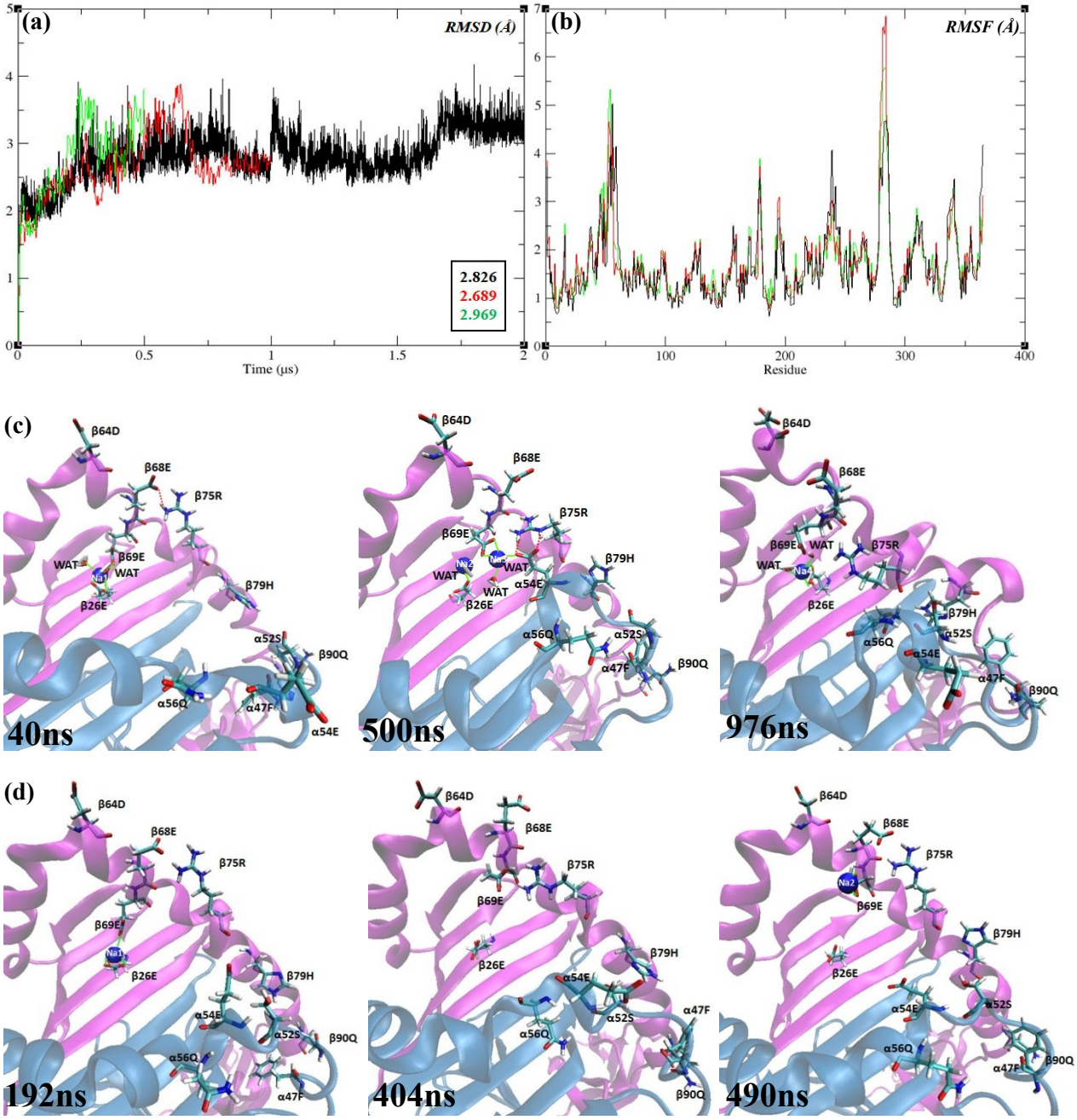


Figure S1. Plot of (a) RMSD taking first frame as reference and (b) RMSF per residue for HLA-DP2 protein for 2μs (black), 1μs (red), 0.5μs (green) simulation run. Inset shows the average RMSD of each simulation run with respective colour codes. Representative snapshots showing the magnified view of peptide binding cleft of HLA-DP2 protein at different intervals of simulation time of (c) 1μs (d) 0.5μs simulation trajectories of HLA-DP2 protein. Structures are generated using VMD.

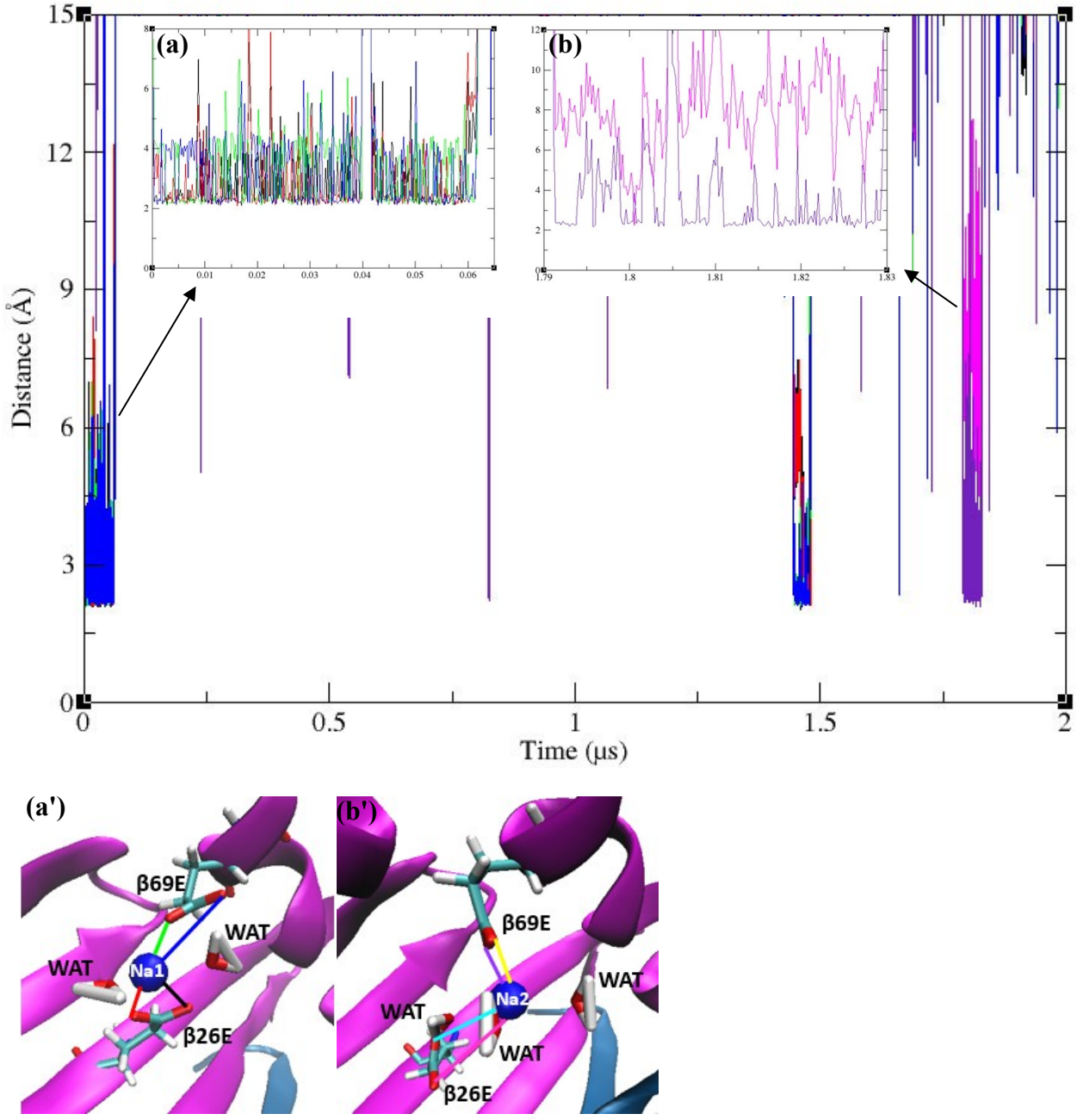


Figure S2. The plot of distances between Na^+ ions with carboxylate oxygens of β26E and β69E of HLA-DP2 protein. Inset shows the zoomed-in section of the areas corresponding to initial (a′) and final (b′) binding sites of Na^+ ions. For the sake of simplicity, the plot of distance limited only to two Na^+ ions coming to the cavity.

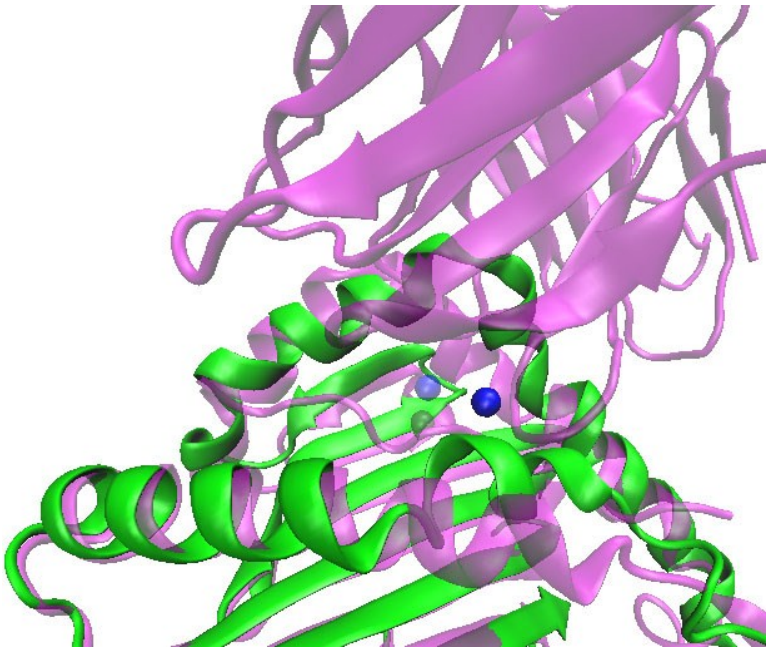


Figure S3. The Superposition of the crystal structure of HLA-DP2_M2_Be_TCR complex (magenta) with average structure of HLA-DP2 protein (green) generated at the time interval 1.4-2μs. The transparent grey and blue spheres indicate Be^{2+} and Na^{+}-ion in the crystal structure and the solid blue sphere indicates Na^{+}-ion in the average simulated structure of apo HLA-DP2.

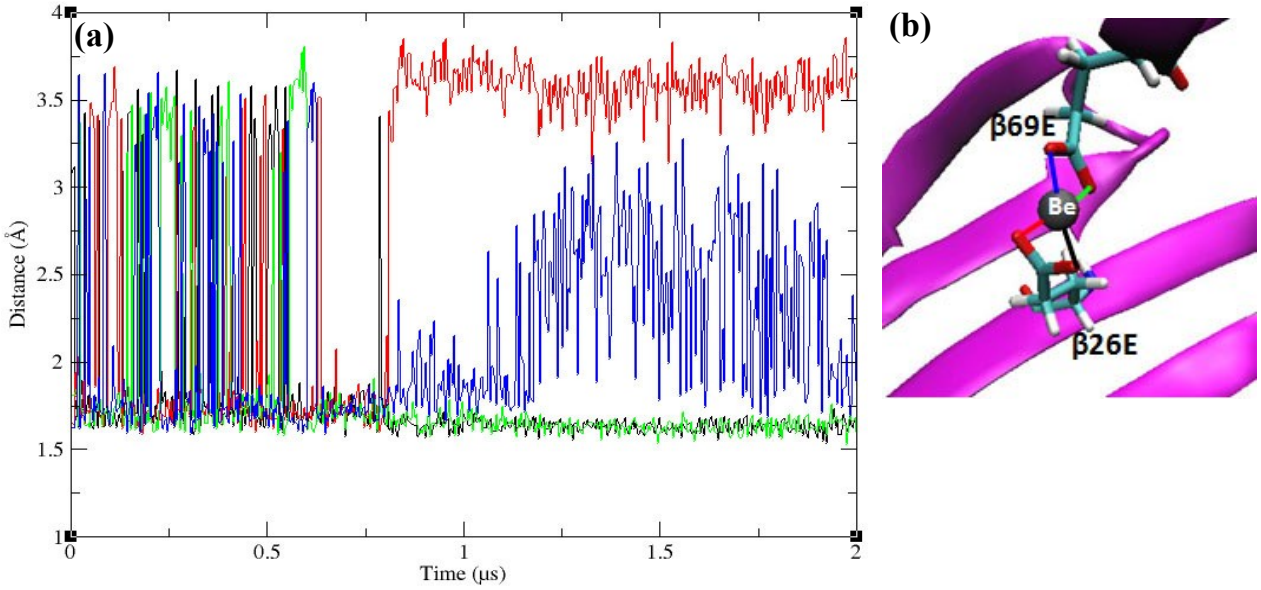


Figure S4. (a) Plot of the distance between Be^{2+} and carboxylate oxygens of β26E (O1: black, O2: red) and β69E (O1: green, O2: blue) versus simulation time for HLA-DP2_Be complex (b) Zoomed-in view of Be^{2+} binding cavity. Glutamate residues are shown in licorice and the distances with Be^{2+} is marked with respective colored lines.

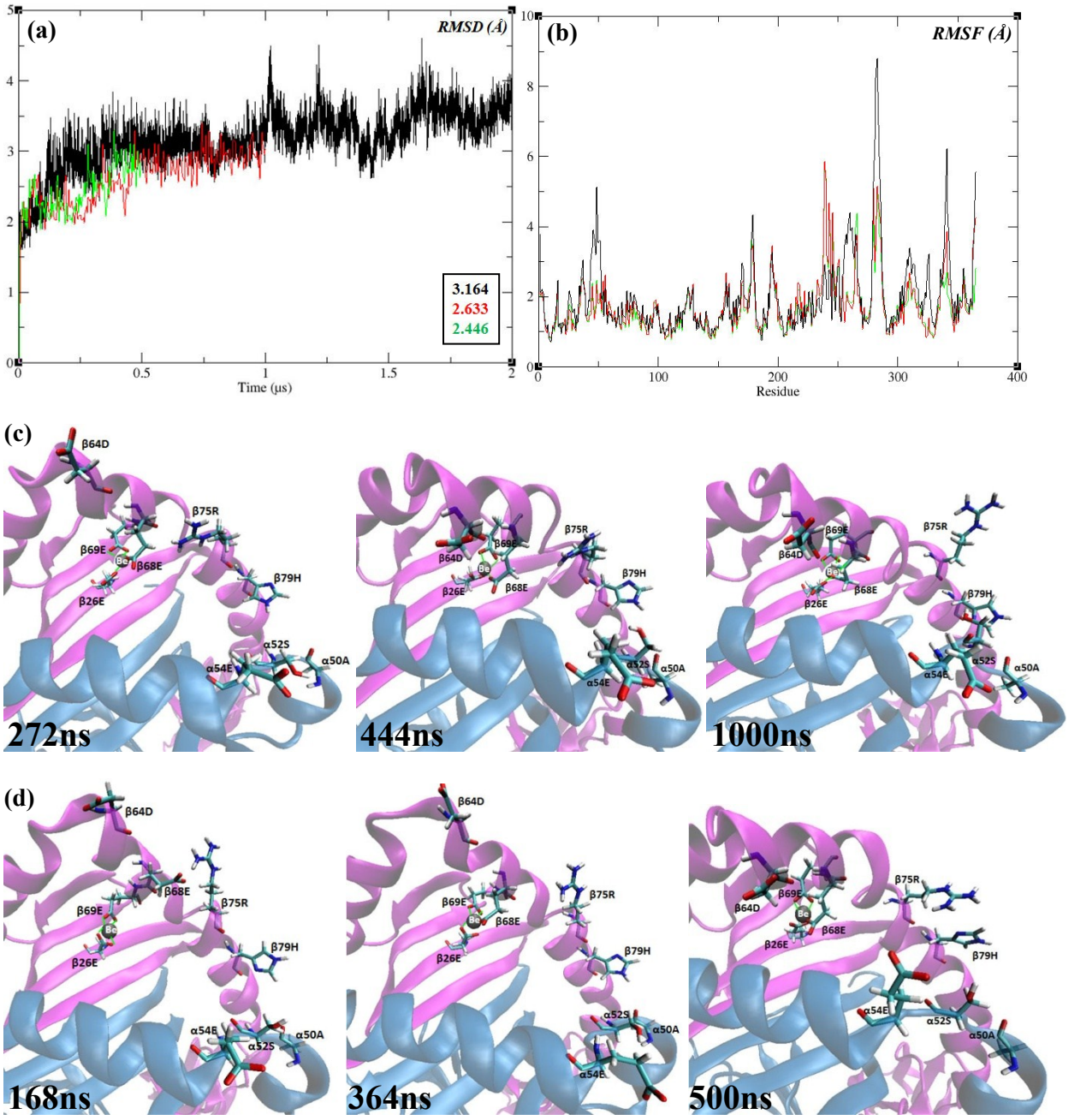


Figure S5. (a) The plot of RMSD with reference to the first frame (b) RMSF per residue for HLA-DP2_Be complex for 2μs (black), 1μs (red), 0.5μs (green) simulation run. Inset shows the average RMSD of each simulation run with respective colour codes. Representative snapshots showing the magnified view of peptide binding cleft of HLA-DP2-Be complex at different intervals of simulation time of (c) 1μs (d) 0.5μs parallel simulation trajectory. Structures are generated using VMD.

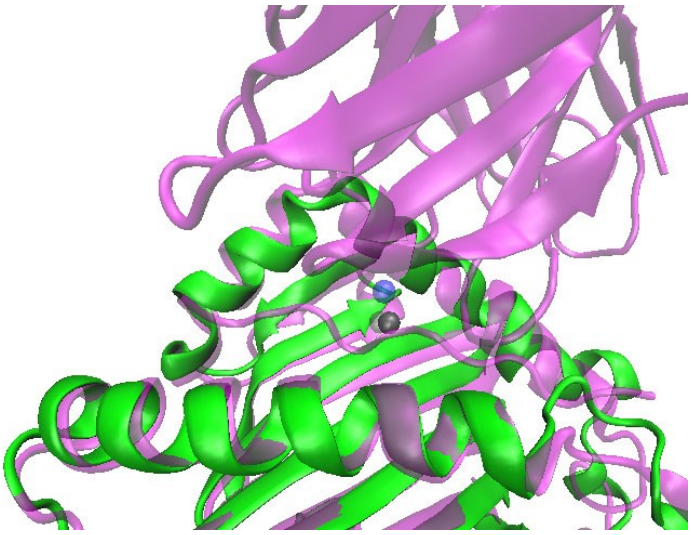


Figure S6. The Superposition of the crystal structure of HLA-DP2_M2_Be_TCR complex (magenta) with average structure of HLA-DP2_Be complex (green) generated at the time interval 1.4-2μs. The transparent grey and blue spheres indicate Be^{2+} and Na^{+}-ion in the crystal structure and the solid grey sphere indicates Be^{2+}-ion in the average simulated structure of HLA-DP2_Be complex.

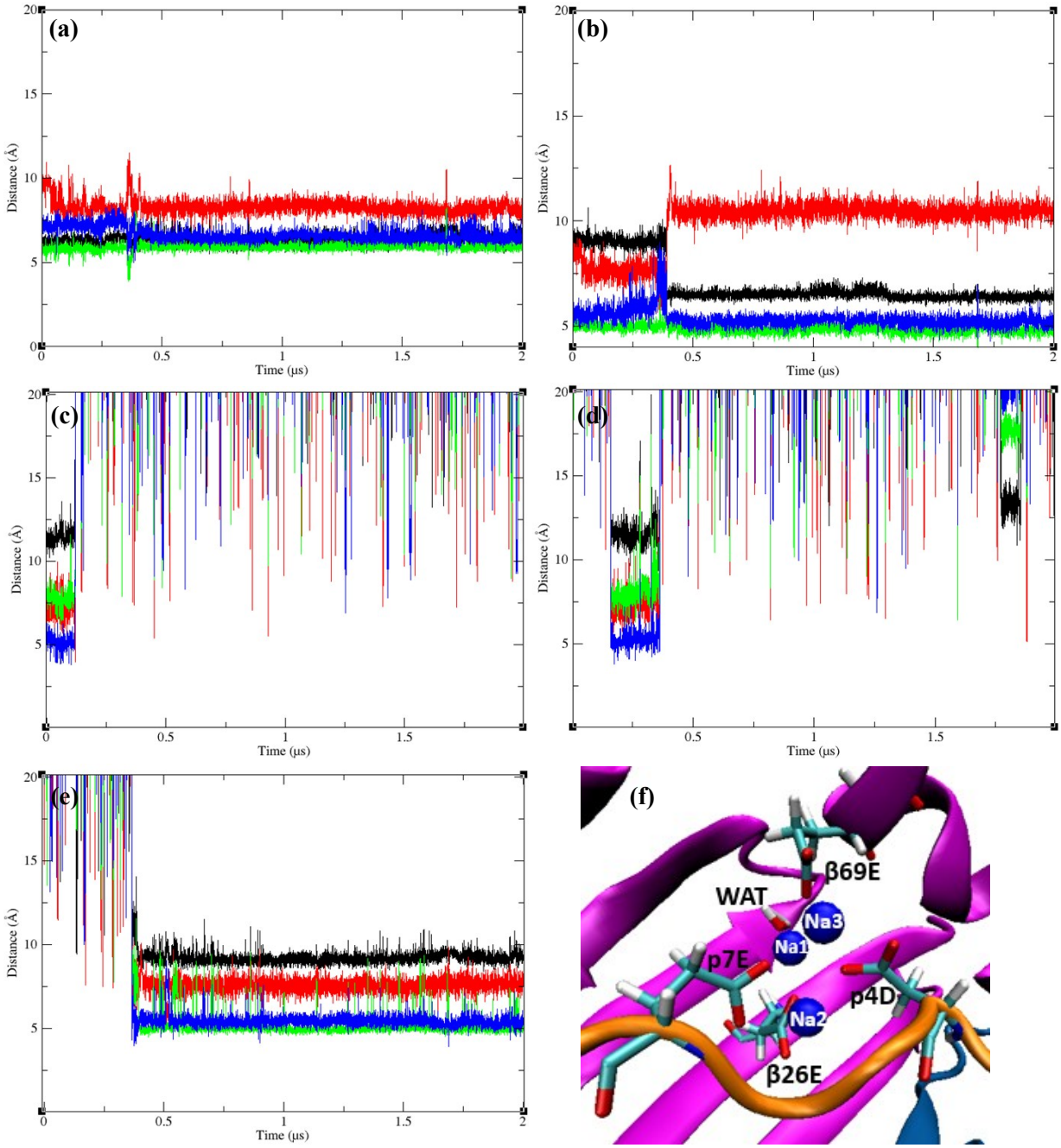


Figure S7. The distances of (a) Na1 (b) Na2 and (c) (d) (e) Na3 ions (ion-exchange) from C_α of β26E (black), β69E (red), p4D (green) and p7E (blue) in the cavity of HLA-DP2_M2 complex for 2μs simulation. (f) Zoomed-in view of HLA-DP2_M2 complex with primary (Na1), secondary (Na2) and tertiary (Na3) binding sites for Na^+ ions.

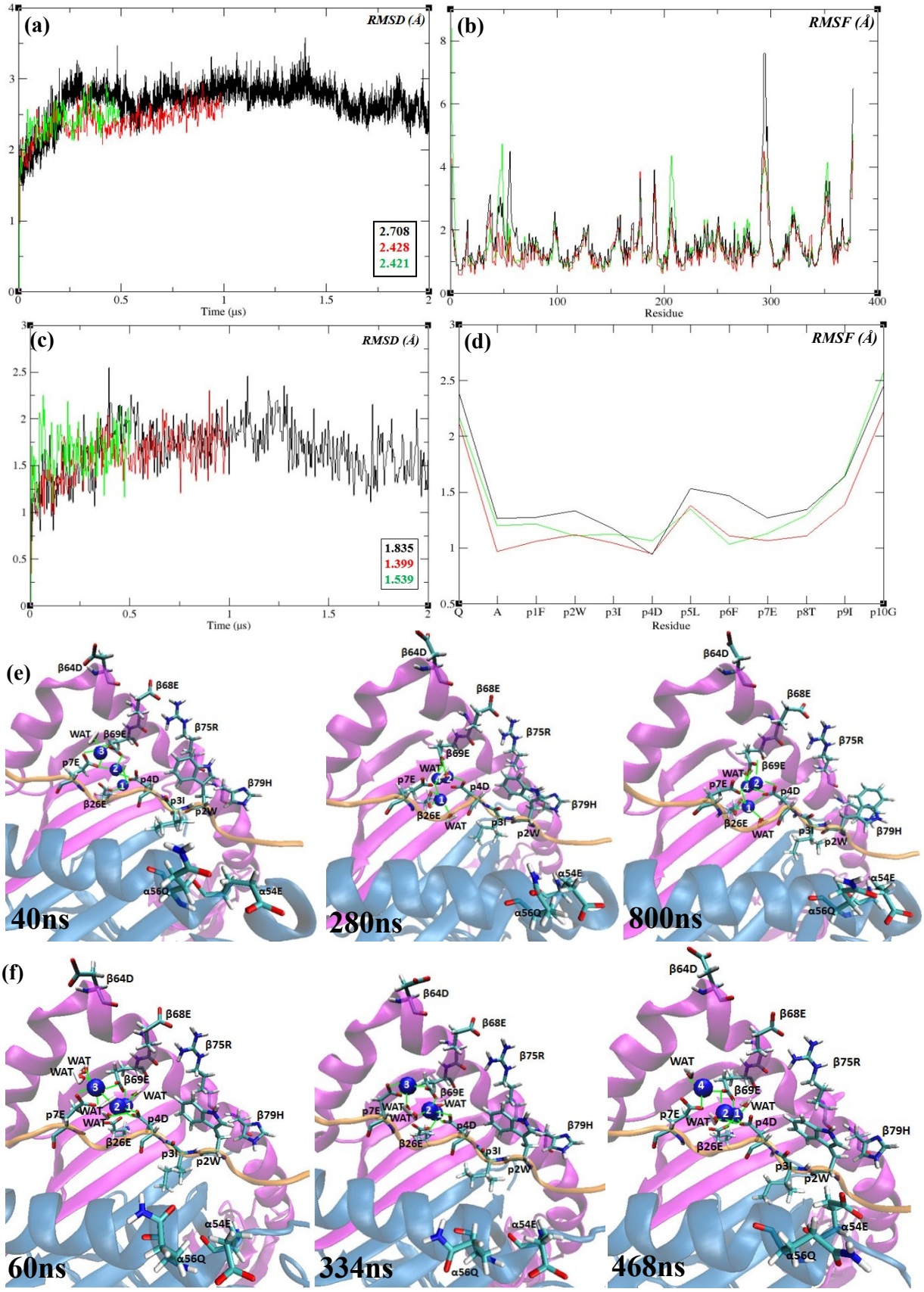

(a)
RMSD (Å)
2.708
2.428
2.421
Time (μs)
(b)
RMSF (Å)
Residue
(c)
RMSD (Å)
1.835
1.399
1.539
Time (μs)
(d)
RMSF (Å)
Q A p1F p2W p3I p4D p5L p6F p7E p8T p9I p10G
Residue
(e)
β64D
β68E
β75R
WAT
β69E
p7E
p4D
β79H
β26E
p3I
p2W
α56Q
α54E
40ns
280ns
800ns
(f)
WAT
60ns
334ns
468ns

Figure S8. The plot of (a) RMSD with reference to the first frame and (b) RMSF per residue for HLA-DP2_M2 complex and the plot of (c) RMSD with reference to the first frame and (d) RMSF per residue for M2 peptide in HLA-DP2_M2 complex for 2μs (black), 1μs (red), 0.5μs (green) simulation run. Inset shows the average RMSD of each simulation run. Representative snapshots showing the magnified view of ion-binding cleft of HLA-DP2_M2 complex at different intervals of simulation time of (e) 1μs (f) 0.5μs simulation trajectories. Structures are generated using VMD.

(a) **(b)**

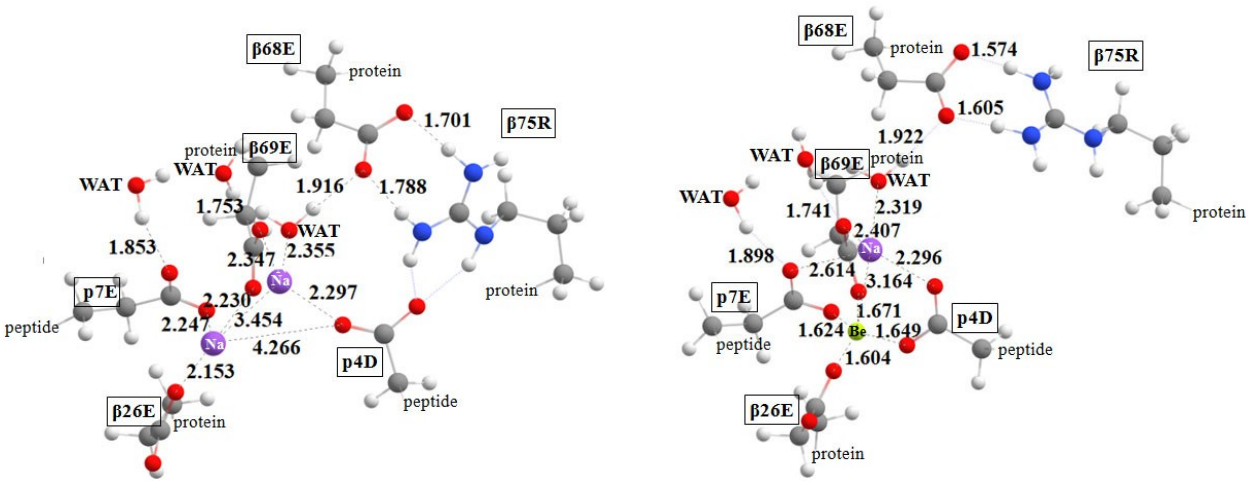


Figure S9. High layer of the optimized geometry of (a) HLA-DP2_M2 complex without Be^{2+} and (b) with Be^{2+} at the ONIOM-EE (B3LYP/6-31+g*:AMBER) level of theory. Atoms are shown in ball and stick and low layer is neglected for the sake of simplicity. Distances are given in Angstroms.

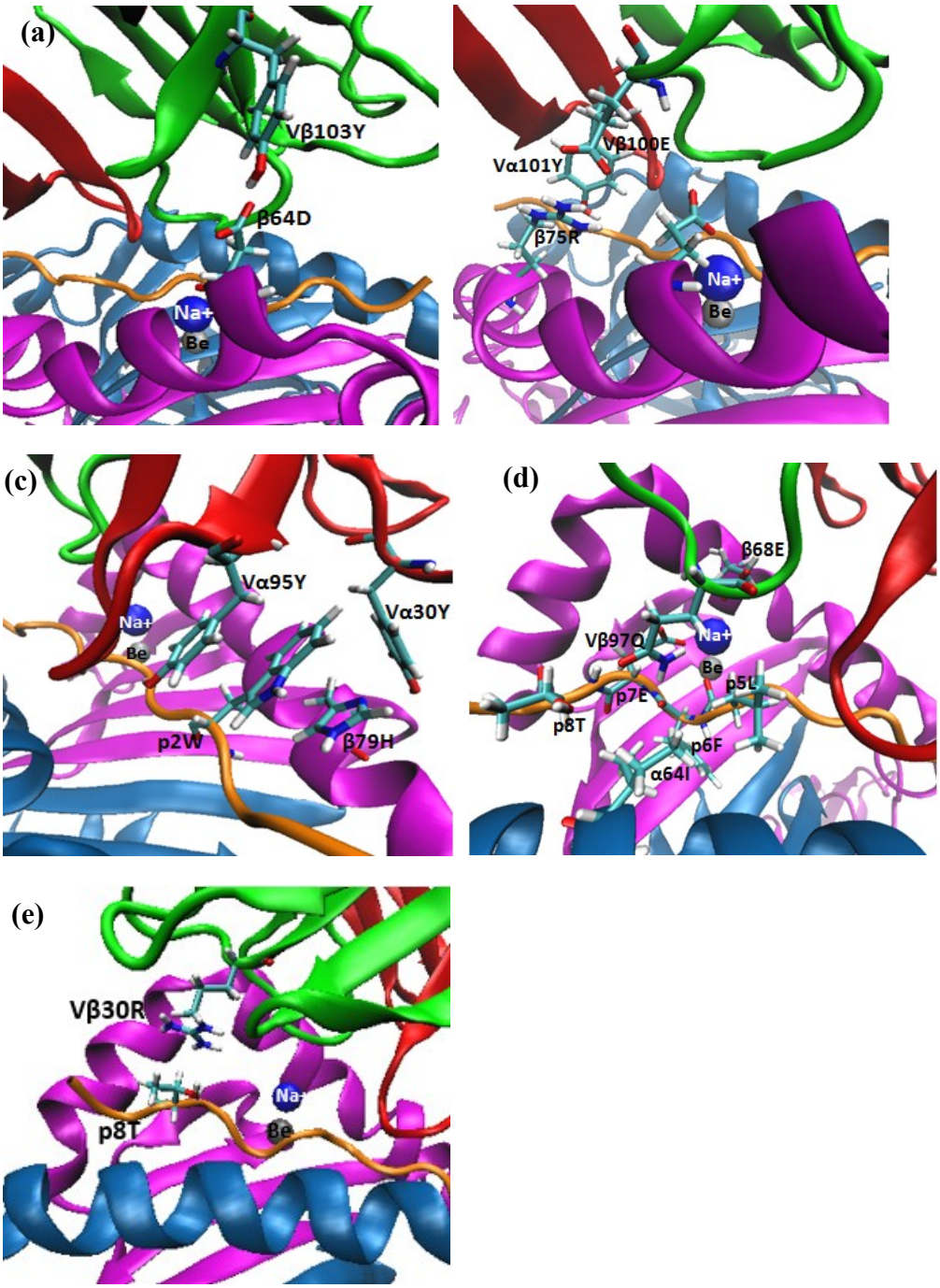


Figure S10. View from top of the binding groove of crystal structure showing interaction between (a) Vβ103Y with β64D (b)Vα101Y and Vβ100E with β75R (c)Vα95Y with p2W and β79H with Vα30Y (d) Vβ97Q and peptide residues (e) Vβ30R with p8T. The HLA-DP2 protein (DP2α: blue, DP2β: magenta), M2 peptide (orange), AV22 TCR (Vα: red, Vβ: green) are rendered as cartoon. The structures are generated using VMD.

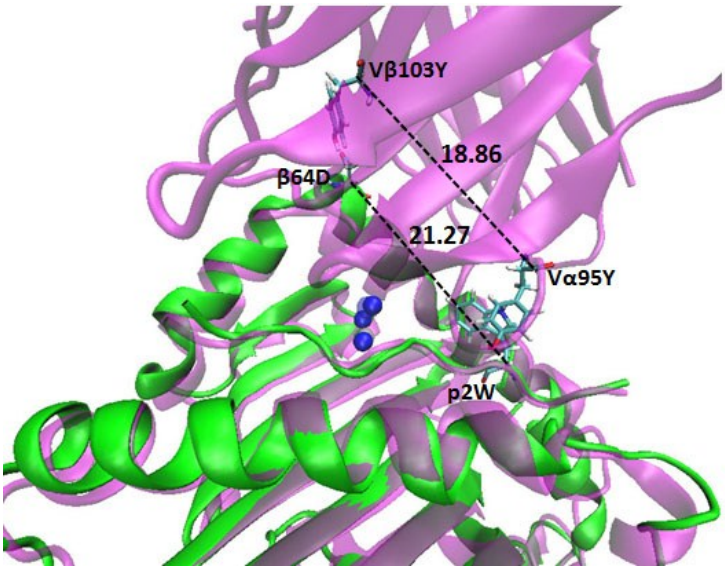


Figure S11. The Superposition of the crystal structure of HLA-DP2_M2_Be_TCR complex (magenta) with average structure of HLA-DP2_M2 complex (green) generated at the time interval 1.4-2 μs. The rmsd of the average structure by taking the crystal structure as reference is 3.071 Å. The transparent and solid blue spheres indicate Na^+-ions in the crystal structure and in the average simulated structure of HLA-DP2_M2 respectively. The important residues are shown in licorice and the distances between the C_α atoms are given in angstrom.

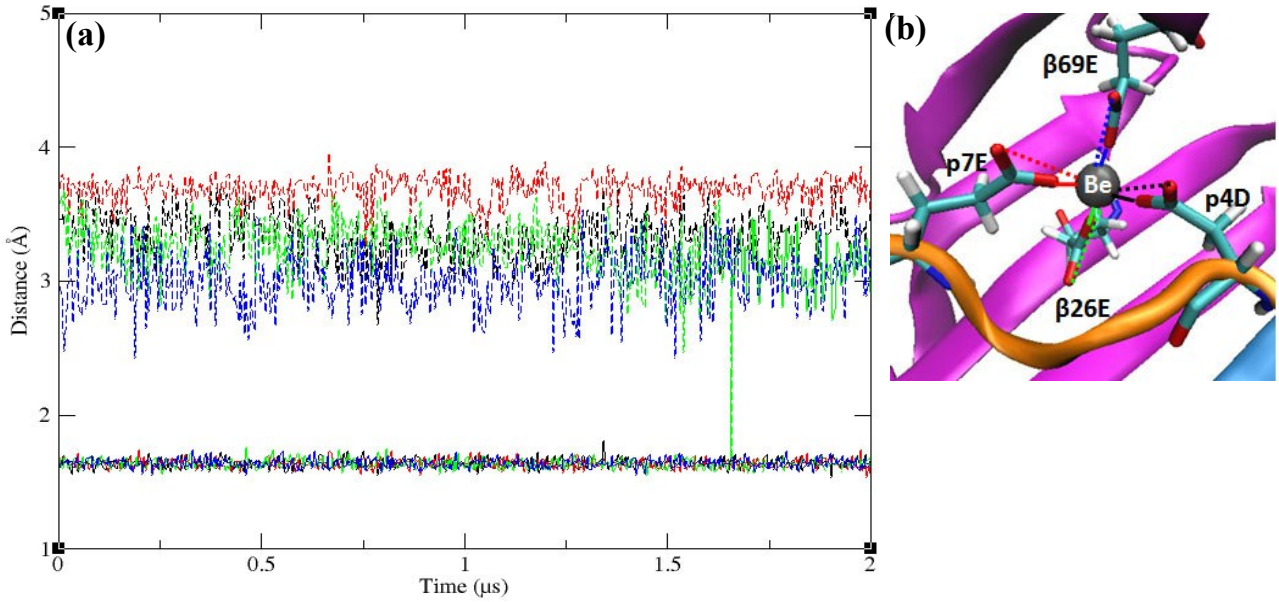
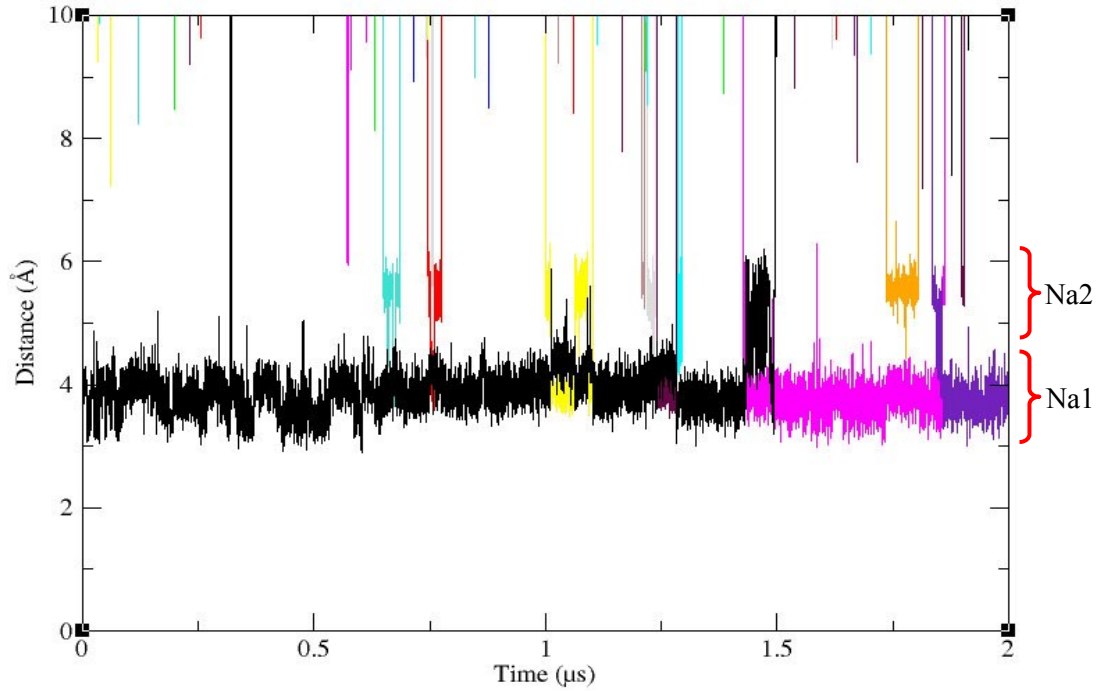


Figure S12. (a) Plot of the distances of Be^{2+} and carboxylate oxygens of amino acid residues β26E (green), β69E (blue), p4D (black) and p7E (red) in the binding cavity of HLA-DP2_M2_Be complex as a function of simulation time. (b) Zoomed-in view of binding site. Solid and dashed lines indicate the distance of Be^{2+} with $O1_{C=O}$ and $O2_{C=O}$ respectively.



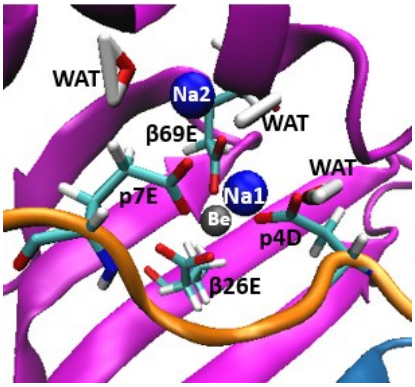


Figure S13. Plot indicates the distances between primary and secondary Na^+ ions with Be^{2+} in the cavity of HLA-DP2_M2_Be complex as a function of simulation time. Each colored line corresponds to different Na^+ ions in the binding cleft. The putative primary and secondary binding site for Na^+ ions is shown with Na1 and Na2 in the zoomed image of binding cavity.

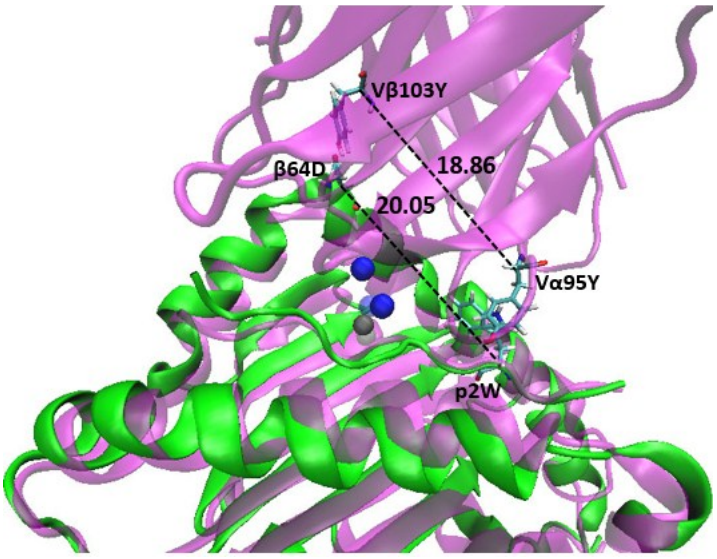


Figure S14. The Superposition of the crystal structure of HLA-DP2_M2_Be_TCR complex (magenta) with average structure of HLA-DP2_M2_Be complex (green) generated at the time interval 1.4-2 μs. The rmsd of the average structure by taking the crystal structure as reference is 2.823 Å. The transparent grey and blue spheres indicate Be^{2+} and Na^{+}-ions in the crystal structure and the solid grey and blue spheres indicate Be^{2+} and Na^{+}-ions in the average simulated structure of HLA-DP2_M2_Be respectively. The important residues are shown in licorice and the distances between the C_{α} atoms are given in angstrom.

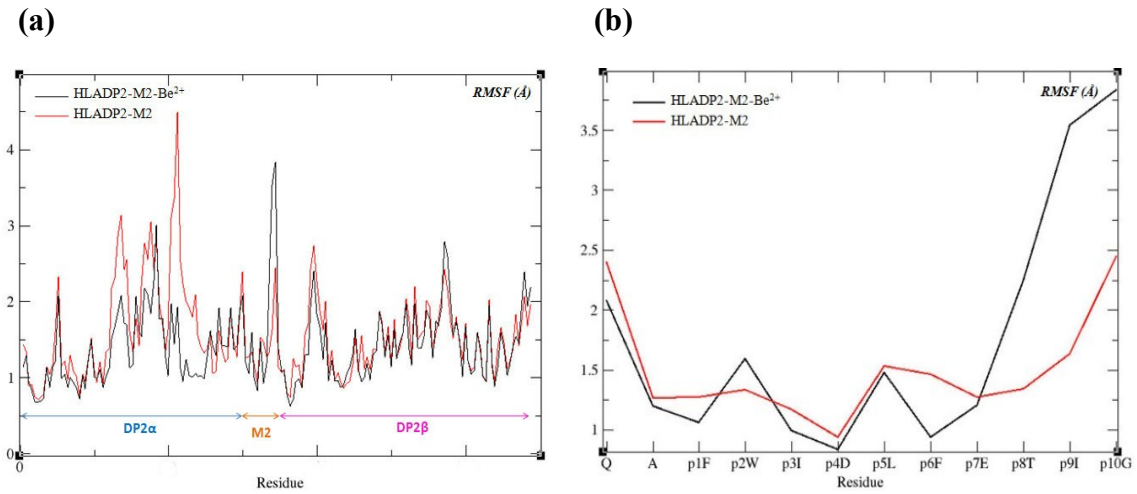


Figure S15. Root-mean square fluctuations (RMSF) computed for 2μs simulation for HLA-DP2_M2_Be (black) and HLA-DP2_M2 (red) complex for (a) all residues and (b) M2 peptide residues.

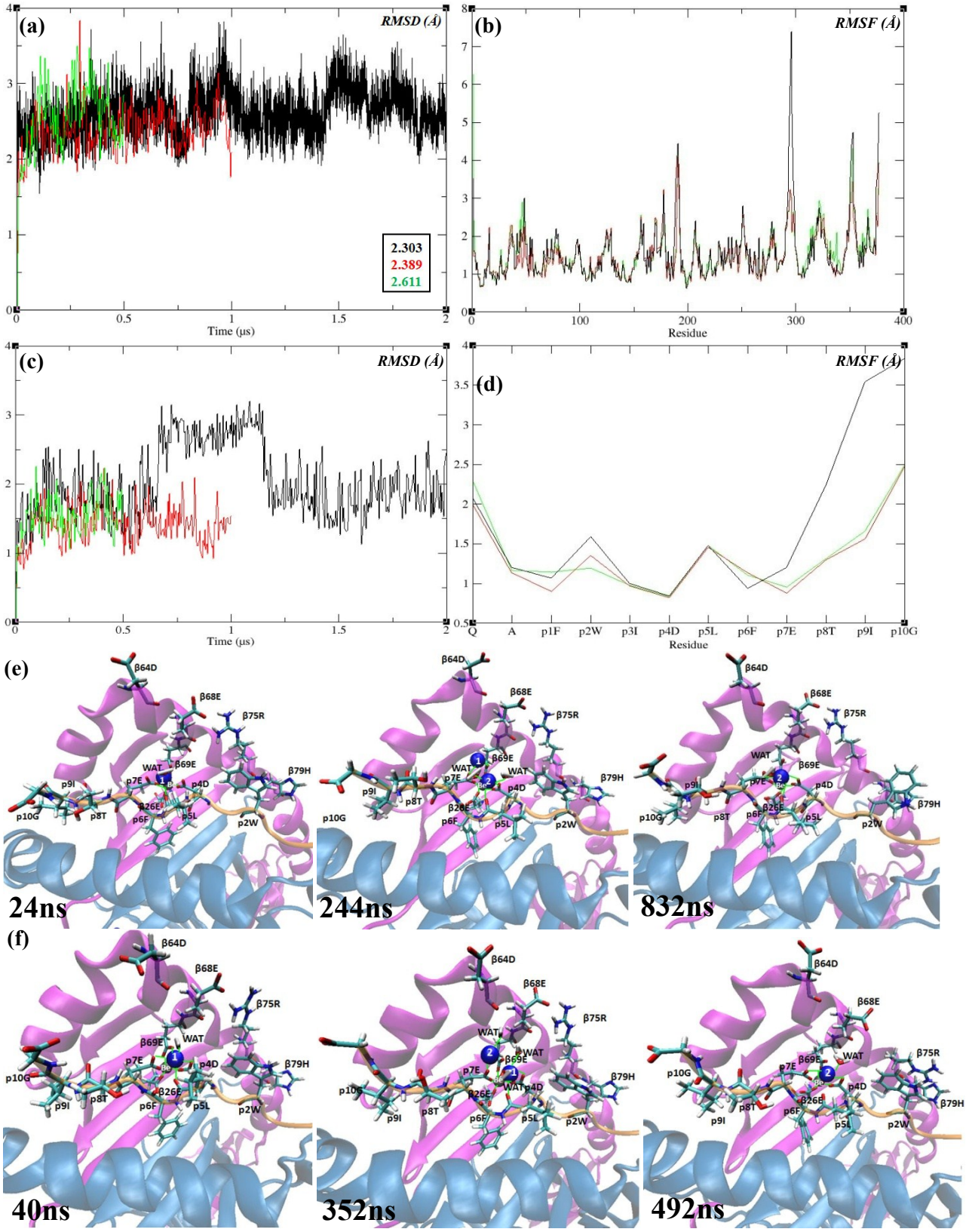

(a)
RMSD (Å)
2.303
2.389
2.611
Time (μs)
(b)
RMSF (Å)
Residue
(c)
RMSD (Å)
Time (μs)
(d)
RMSF (Å)
Q A p1F p2W p3I p4D p5L p6F p7E p8T p9I p10G
Residue
(e)
β64D β68E β75R β69E WAT β79H p9I p7E p4D p10G p8T β26E p6F p5L p2W
24ns
244ns
832ns
(f)
40ns
352ns
492ns

Figure S16. (a) The plot of RMSD with reference to the first frame and (b) RMSF per residue for HLA-DP2_M2_Be complex and (c) The plot of RMSD with reference to the first frame and (d) RMSF for M2 peptide of HLA-DP2_M2_Be complex for 2μs (black), 1μs (red), 0.5μs (green) simulation run. Inset shows the average RMSD of each simulation run. Representative snapshots showing the magnified view of binding cleft of HLA-DP2_M2_Be complex at different intervals of simulation time of (e) 1μs (f) 0.5μs parallel simulation trajectory. Structures are generated using VMD.

Table S1. The calculated pKa values of Aspartic acid (D) and Glutamic acid (E) residues at pH = 7.0 using the DelPhiPKa Web server.

	pKa	
Residue	**HLA-DP2_M2**	**HLA-DP2**
α 3D	3.52	3.52
α 20E	3.69	3.67
α 24E	4.34	4.31
α 26D	4.16	4.14
α 27E	4.26	4.23
α 28D	3.23	3.22
α 29E	4.40	4.39
α 34D	3.42	3.42
α 36D	3.59	3.59
α 39E	3.84	3.82
α 45E	4.02	4.02
α 46E	3.87	3.86
α 54E	3.92	3.89
α 84D	3.61	3.61
α 87E	3.83	3.83
α 94E	4.00	4.00
α 97E	4.06	4.06
α 109D	2.85	2.86
α 125E	4.15	4.15
α 129E	4.01	4.01
α 133E	3.88	3.88
α 141D	2.42	2.42
α 157E	4.10	4.10
α 158D	4.15	4.15
α 161D	3.88	3.88
α 165E	3.76	3.76
α 170D	3.95	3.95
α 178E	4.07	4.07
p4D	4.40	-
p7E	5.28	-
β 5E	4.07	4.07
β 14E	2.51	2.52
β 26E	4.59	4.11
β 33E	3.93	3.92
β 34E	2.84	2.85
β 39D	2.07	2.07
β 41D	3.55	3.54
β 44E	3.38	3.37
β 50E	3.98	3.98
β 55D	2.48	2.54
β 56E	3.37	3.36
β 57E	3.60	3.60
β 64D	3.87	3.86
β 67E	3.59	3.56

β 68E	4.31	4.03
β 69E	5.30	4.22
β 74D	3.60	3.58
β 82E	3.92	3.89
β 119D	3.83	3.83
β 135E	3.87	3.87
β 136E	4.12	4.12
β 150D	3.34	3.34
β 160E	4.01	4.01
β 167D	3.84	3.84
β 174E	3.94	3.94
β 179D	3.90	3.90
β 185E	3.96	3.96

Table S2. The calculated binding free energies using MM-PBSA method.

	Ligand	
Receptor	**Be^{2+}**	**Na^{+}**
HLA-DP2	-143.3	-13.3
HLA-DP2_M2	-401.2	-6.9
	M2	**RAS**
HLA-DP2	+43.4	-80.9
HLA-DP2_Na^{+}	-38.5	-
HLA-DP2_3Na^{+}	-129.0	-
HLA-DP2_Be^{2+}	-174.8	-
HLA-DP2_Be^{2+}_2Na^{+}	-211.7	-

Energy values given in kcal/mol.
*deformed protein

Table S3. Energy Decomposition Analysis of the ONIOM optimized geometry of HLA-DP2_M2 with Be^{2+} and Na^{+} at the BP86/TZ2P level.

	Be^{2+}	**Na^{+}**
ΔE_{int}	-1192.63	-372.00
ΔE_{Pauli}	70.22	33.70
ΔE_{elstat}	-857.55 (67.91%)	-370.88 (91.41%)
ΔE_{orb}	-405.31 (32.09%)	-34.82 (8.58%)

Energy values given in kcal/mol. The value in parentheses gives the percentage contribution towards total attractive interaction.