Molecular Dynamics Simulations to Identify the Binding Hot Spots of the HIV-1 Coat Protein GP41 and Broadly Neutralizing Antibody 10E8

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10E8 is one of the most potent HIV-neutralizing antibodies isolated and it neutralizes up to 98% of diverse HIV–1 strains [1]. 10E8 is specific to the membrane-proximal external region (MPER) of the HIV envelope protein gp41 and 10E8 is orthogonal to other anti-HIV antibodies [1]. In combination with other antibodies 10E8 may provide an antibody response that neutralizes nearly all strains of HIV–1 [1]. Additionally, 10E8 effectively induces antibody-dependent cellular cytotoxicity indicating its potential use for therapeutic vaccine strategies [1]. Further, 10E8 is a tool for immunogen design and validation of immunogen structure [2].

To explore the mechanism of HIV-1 neutralization by 10E8 and thus obtain valuable information for vaccine and drug design, molecular dynamics (MD) simulations and binding free energy calculations were performed in this study for crystal structure of 10E8 Fab in complex with its gp41 MPER epitope [1].

The MD simulations were carried out by the Amber 11 computer package using the Amber ff10 force field [3]. The X-ray structure of the gp41/10E8 complex [1] was placed in a truncated octahedron box with walls at least 10 Å from the nearest structural atoms, filled with TIP3P water [4] as an explicit solvent, and subjected to periodic boundary conditions. The system was prepared for MD simulation by 500 steps of steepest descent followed by

1000 steps of conjugate gradient energy minimization. The atoms of the assembly were then fixed by an additional harmonic potential with the force constant equal to 1.0 kcal/mol and then heated from 0 to 310 K over 1.4 ns using a constant volume of the unit cell. Additional equilibration was performed over 1 ns by setting the system pressure to 1.0 atm and by using a weak coupling of the system temperature to a 310 K bath [5] with a 2.0 ps characteristic time. Finally, the constraints on the complex were removed and the system was equilibrated again at 310 K over 2 ns under constant volume conditions. After equilibration, the isothermal-isobaric MD simulation (T = 310 K, P = 1.0 atm) generated 60 ns trajectory for the gp41/10E8 complex using a Berendsen barostat with a 2.0 ps characteristic time, a Langevin thermostat [3] with collision frequency of 2.0 ps^{-1}, a non-bonded cut-off distance of 8 Å, and a simple leapfrog integrator [3] with a 2.0 fs time step and hydrogen atoms constrained by the SHAKE algorithm [6]. Electrostatic interactions were calculated at every step with the particle-mesh Ewald method [7], short-range repulsive and attractive dispersion interactions were simultaneously described by a Lennard-Jones potential.

The MM-PBSA method [8] was used to calculate the binding free energy and to analyze the binding interaction in detail. The polar solvation energies were computed in continuum solvent using Poisson-Boltzmann and ionic strenght of 0.1. The non-polar terms were estimated using solvent accessible surface areas [9]. 2000 snapshots were selected from the last 20 ns, by keeping the snapshots every 10 ps. The ptraj procedure associated with AMBER 11 [3] was used to identify hydrogen bonds in the dynamic structures of the complex of interest.

By decomposing the binding free energy into the contribution from each residue, the binding hot spots for 10E8 and gp41 were identified (Table 1). For 10E8, the heavy chain residues Tyr-99, Asp-100, Phe-100a and Trp-100b provide significant contributions (Table 1). For gp41, residues Trp-672, Phe-673 and Arg-683 were identified as hotspots (Table 1), in agreement with the data on alanine scanning and paratope analysis [1] according to which these residues of the gp41 MPER region are of great importance to its specific interactions with 10E8. The findings obtained are also in line with the X-ray data [1], whereby the most part of the above residues of 10E8 and gp41 is involved in direct intermolecular contacts resulting in the formation of stable supramolecular structure. With the data of a study [1],

Phe-100a and Gly-100d of 10E8 form hydrogen bonds with Arg-683 of gp41. At the same time, Trp-33 and Tyr-99 of 10E8 make van der Waals contacts with Trp-672 of gp41, and antibody residue Trp-100b comes in van der Waals interaction with gp41 amino acid Arg-683. In addition, this interaction mode also appears between functionally important Phe-673 of gp41 and Arg-95b of 10E8 that also contributes to the binding enthalpy (Table 1).

Table 1. Mean values of the binding enthalpy for amino acid residues of 10E8 and gp41

Amino acid of 10E8 [a]	Enthalpy of binding [b] kcal/mole	Amino acid of gp41	Enthalpy of binding [b] kcal/mole
Asn-31^{H}	-0.54	Leu-660	-3.08
Trp-33 ^{H}	-2.03	Leu-663	-1.31
Pro-52b ^{H}	-0.63	Asn-671	-3.19
Gly-52c ^{H}	-1.39	Trp-672	-7.61
Tyr-98 ^{H}	-1.04	Phe-673	-5.03
Tyr-99 ^{H}	-5.67	Thr-676	-1.54
Asp-100 ^{H}	-3.86	Leu-679	-2.36
Phe-100a ^{H}	-6.08	Trp-680	-0.50
Trp-100b ^{H}	-4.20	Arg-683	-6.01
Gly-100d ^{H}	-2.51	Arg-684	-2.45
Pro-100f ^{H}	-1.09	–	–
Glu-53^{L}	-1.22	–	–
Arg-95b ^{L}	-1.25	–	–

Footnotes: [a] Superscripts H and L indicate amino acids associated with the 10E8 heavy and light chains respectively. [b] The data for amino acid residues with the binding enthalpy ≤ -0.5 kcal/mole are presented.

The MD simulations support the X-ray data of a study [1]. Analysis of the MD structures indicates that intermolecular hydrogen bonds play an important role in the binding, involving residues of gp41 and 10E8 that dominate the complex formation (Table 1). In particular, Arg-683 of gp41 and Asp-100 of 10E8 make two hydrogen bonds with occupancy values exceeding 83% and 22%. In addition, Trp-672 of gp41 and Glu-53 of 10E8 participate in the H-bonding with occupancy of more than 74%. Along with hydrogen bonds, the MD structures exhibit a great number of van der Waals contacts associated with the residues of

10E8 and gp41 that are responsible for the energy stabilization of the gp41/10E8 complex (Table 1).

Thus, the MD simulations of the crystal structure of 10E8 Fab in complex with the HIV-1 gp41 MPER region show that residues Tyr-99, Asp-100, Phe-100a, Trp-100b of the antibody heavy chain and Trp-672, Phe-673, Arg-683 of gp41 are critical for the binding. These findings provide some useful insights that should aid the design of vaccines and small molecule inhibitors to neutralize HIV-1.

References

1. J. Huang, G. Ofek, L. Laub et al. (2012) Broad and potent neutralization of HIV-1 by a gp41-specific human antibody, *Nature,* **491:** 406–412.

2. M.J. van Gils, R.W. Sanders (2013) Broadly neutralizing antibodies against HIV-1: Templates for a vaccine. *Virology*, **435:** 46–56.

3. D.A. Case, T.E. Cheatham, C.L. Simmerling et al. (2010) AMBER 11, University of California, San Francisco.

4. W.L. Jorgensen, J. Chandrasekhar, J.D. Madura et al. (1983) Comparison of simple potential functions for simulating liquid water, *J. Chem. Phys.,* **79:** 926–935.

5. H.J.C. Berendsen, J.P.M. Postma, W.F. van Gunsteren et al. (1984) Molecular dynamics with coupling to an external bath, *J. Chem. Phys.,* **81:** 3684–3690.

6. J.P. Ryckaert, G. Ciccotti, H.J.C. Berendsen (1977) Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes, *J. Comput. Phys.,* **23:** 327–341.

7. U. Essmann, L. Perera, M.L. Berkowitz et al. (1995) A smooth particle mesh Ewald method, *J. Chem. Phys.,* **103:** 8577–8592.

8. I. Massova, P.A. Kollman (1999) Computational alanine scanning to probe protein-protein interactions: a novel approach to evaluate binding free energies, *J. Amer. Chem. Soc.,* **121:** 8133–8143.

9. K. Lindorff-Larsen, S. Piana, K. Palmo et al. (2010) Improved side-chain torsion potentials for the Amber ff99SB protein force field, *Proteins,* **78:** 1950–1958.