Supplementary Materials

Investigation of Flap Flexibility of β -Secretase Using Molecular Dynamic Analyses

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Setting up simulations

The MD simulations were executed using the GPU version of the PMEMD engine provided with the Amber 14 package (1-3). The FF99SB variant of the AMBER force field (4) was used to describe the protein. The restrained electrostatic potential (RESP) procedure (5) was used to calculate the partial atomic charges for the ligands at the HF/6-31G* level using the Gaussian 03 package (6). The LEAP module in Amber 14 was used for addition of hydrogen atoms to the protein and the addition of counter ions for neutralization. The systems were embedded within an orthorhombic box of TIP3P (7) water molecules such that no protein atom was within 10 Å of any box edge. Periodic boundary conditions were enforced and the long-range electrostatic interactions were treated with the particle-mesh Ewald (PME) method (8) assimilated in Amber14 with a direct space and vdW cut-off of 12 Å. Initial energy minimization of each system, with a restraint potential of 500 kcal/mol Å² applied to the solute, was performed for 1000 steps of steepest descent followed by 1000 steps of conjugate gradient minimization. A further 1000 steps of unrestrained conjugate gradient minimization of the entire system was then carried out. Canonical ensemble (NVT) MD simulations were then carried out for 50 ps, with gradual heating from 0 to 300K with harmonic restraints of 5 kcal/mol Å² applied to all solute atoms and a Langevin thermostat with a random collision frequency of 1/ps. The systems were subsequently equilibrated at 300 K in the NPT ensemble for 5ns, during which no restrains were imposed and a Berendsen barostat was used to maintain the pressure at 1 bar. The SHAKE algorithm (9) was used to constrain the bonds of all hydrogen atoms, a time step of 2 fs and the SPFP precision model(10, 11) was used for all MD runs. Finally a production run of 100ns each was performed in an isothermal isobaric (NPT) ensemble using a Berendsen barostat (12) with a target pressure of 1 bar and a pressure coupling constant of 2 ps. Coordinates were saved every 1 ps and the trajectories were analysed every 1 ps using the CPPTRAJ module integrated with Amber 14.

System Id	Total Atom Numbers
BACE _{Free}	50187
BACE _{Bound1}	53026

Figure S1. The C- α RMSD fluctuation for BACE_{Apo}, BACE_{Bound1} and BACE_{Bound2} during simulation time.

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