Supporting Information

Cocaine Esterase-Cocaine Binding Process and the Free Energy Profiles by Molecular Dynamics and Potential of Mean Force Simulations

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Supporting Information Available. Figure S1 for the experimental kinetic data obtained for (-)-cocaine hydrolysis catalyzed by the L119A/L169K/G173Q mutant of CocE, Figure S2 for the plots of the RMSD of the MD-simulated CocE-(-)-cocaine complexes, Figure S3 for the free energy profiles of the T172R/G173Q CocE binding with (-)-cocaine determined by using two different lengths of the MD trajectory, Figure S4 for the plot of a key distance in the simulated T172R/G173Q CocE-(-)-cocaine binding structure versus the reaction coordinate of the PMF simulations, and Figure S5 for typical snapshot structures of the simulated enzyme-(-)-cocaine complexes along the reaction coordinate. These materials are available free of charge via the Internet http://pubs.acs.org.

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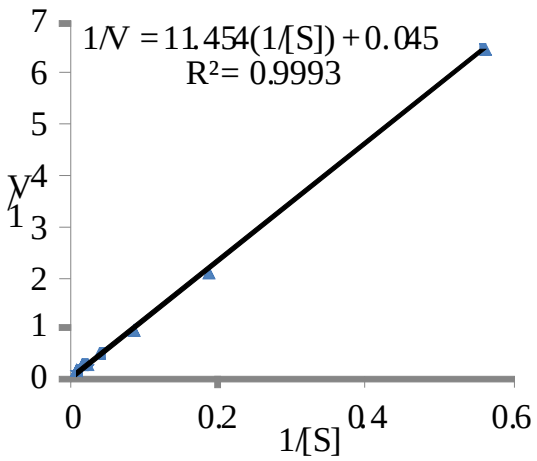

Figure S1. Double-reciprocal plot of the enzyme kinetics for (-)-cocaine hydrolysis catalyzed by the L119A/L169K/G173Q mutant of CocE. [S] represents the initial concentration of (-)-cocaine in μM and V refers to the relative initial rate of the enzymatic hydrolysis of (-)-cocaine.

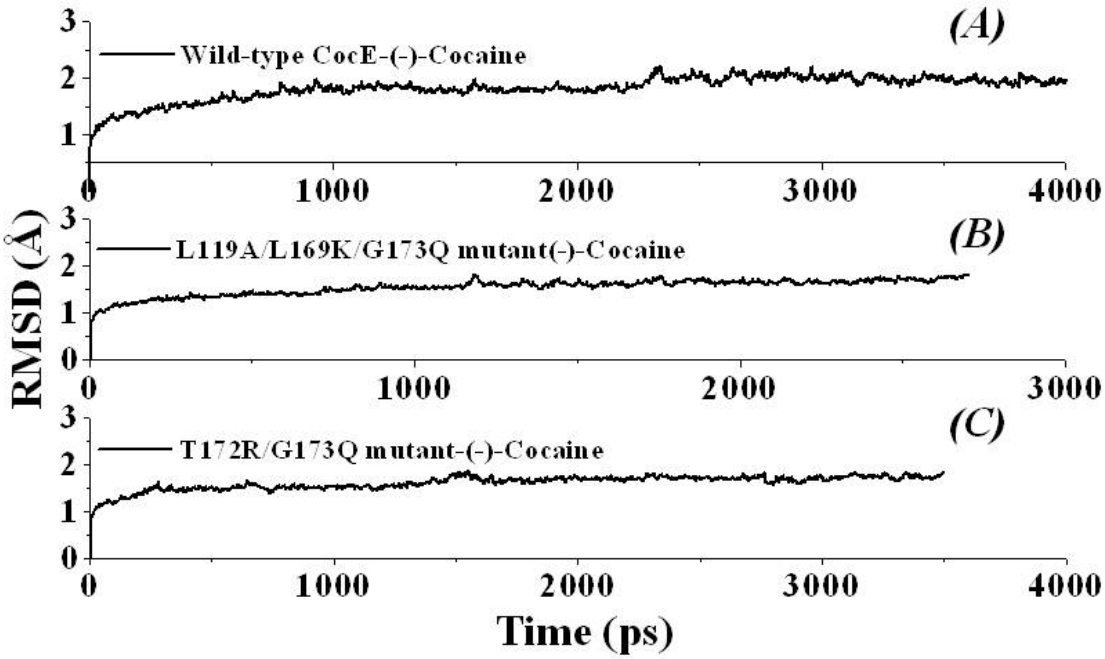

Figure S2. Tracked positional root-mean square deviation (RMSD) for MD simulated three structures of CocE-(-)-cocaine complexes along the MD trajectories. The RMSD was calculated for all the non-hydrogen atoms of each CocE-(-)-cocaine complex using the starting structure as a reference. (A) RMSD for wild-type CocE-(-)-cocaine binding structure; (B) RMSD for the L119A/L169K/G173Q CocE-(-)-cocaine binding structure; and (C) RMSD for the T172R/G173Q CocE-(-)-cocaine binding structure.

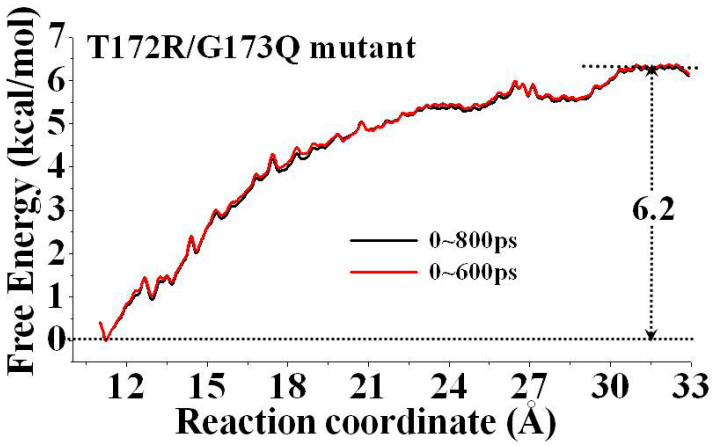

Figure S3. Free energy profile for T172R/G173Q CocE binding with (-)-cocaine determined by using different length of MD trajectory from each window during the PMF simulations, *i.e.* the black curve is determined by using 0.2-1.0 ns MD trajectory from each window, the red curve is determined by using 0.2-0.8 ns MD trajectory from each window. The binding free energy difference from using different length of MD trajectory is only 0.04 kcal/mol. The reaction coordinate was defined as the distance between the mass center of the non-hydrogen atoms of (-)-cocaine and the mass center of the non-hydrogen atoms on the side chains of residues H87, V121, and L146 of the enzyme.

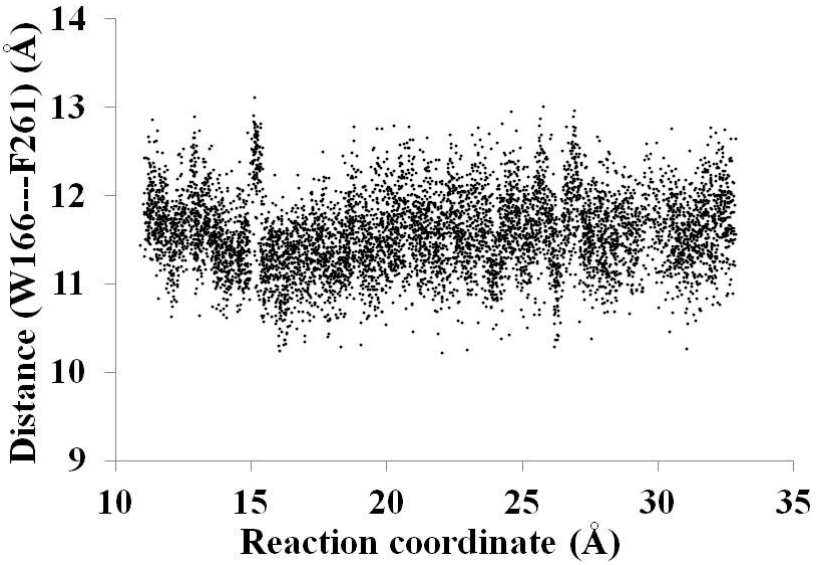

Figure S4. For T172R/G173Q CocE-(-)-cocaine binding structure, the tracked distance between the mass center of W166 side chain and the mass center of F261 side chain based on the last 100 ps MD trajectory from each window along the reaction coordinate of PMF simulations. We took a snapshot for each picosecond, and each dot on the figure refers to the distance in a snapshot. The reaction coordinate was defined as the distance between the mass center of the non-hydrogen atoms of (-)-cocaine and the mass center of the non-hydrogen atoms on the side chains of residues H87, V121, and L146 of the enzyme.

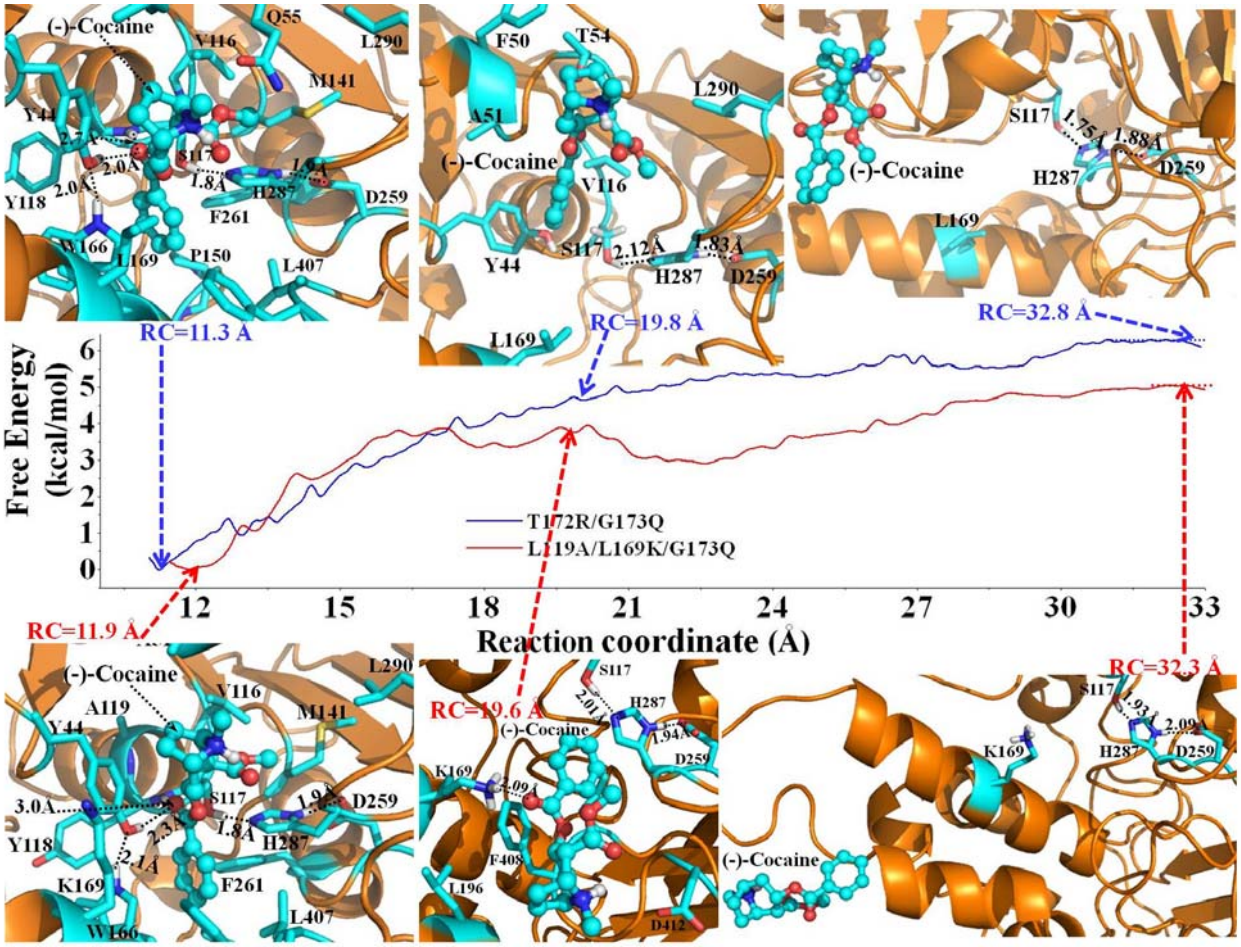

Figure S5. Typical structures of the PMF-simulated enzyme-(-)-cocaine complexes along the reaction coordinate. CocE is shown as gold ribbons and (-)-cocaine is in ball-and-stick style. **Upper panel**: structures of the T172R/G173Q CocE-(-)-cocaine binding complex from the snapshots (0 ps of PMF window #1, 1000 ps of window #27, and 900 ps of window #70) of the MD trajectories along the reaction coordinate. Important residues of CocE are shown in stick style and colored by atom types. Key distances are indicated with dashed lines and labeled, also indicated the values of the reaction coordinate (RC). **Bottom panel**: structures of the L119A/L169K/G173Q CocE-(-)-cocaine binding complex from the snapshots (0 ps of window #1, 291 ps of window #26, 1000 ps of window #70) of the MD trajectories along the reaction coordinate. Important residues of CocE are represented in stick style and colored in atom types. Dashed lines and the labels represent the important distances including that for the hydrogen bonding between the K169 side chain and carbonyl oxygen atom at the benzoyl group of (-)-cocaine. The values of the reaction coordinate (RC) are also indicated.