Supporting Information

Human Butyrylcholinesterase-Cocaine Binding Pathway and Free Energy Profiles by Molecular Dynamics and Potential of Mean Force Simulations

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Supporting Information Available. Additional three figures (Figures S1 to S3) for the ES1-like binding structures and the free energy profiles of the ES binding processes and five tables (Tables S1 to S5) for the structural changes during the ES binding process. This material is available free of charge via the Internet http://pubs.acs.org.

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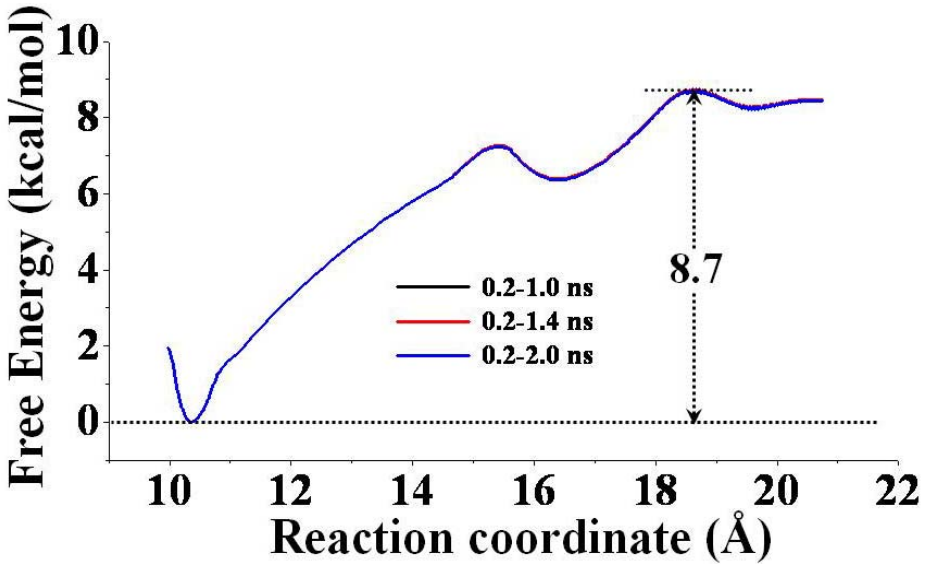

Figure S1. PMF-simulated free energy profile for **wild-type BChE binding with (+)-cocaine**. The black curve was determined by using 0.2-1.0 ns MD trajectory from each window, the red curve was determined by using 0.2-1.4 ns MD trajectory from each window, and the blue curve was determined by using 0.2-2.0 ns MD trajectory from each window. The three curves are nearly identical or indistinguishable. The reaction coordinate was defined as the distance between the mass center of all non-hydrogen atoms of either (-)-cocaine or (+)-cocaine and the mass center of all non-hydrogen atoms belonging to the E197 and I442 side chains of BChE.

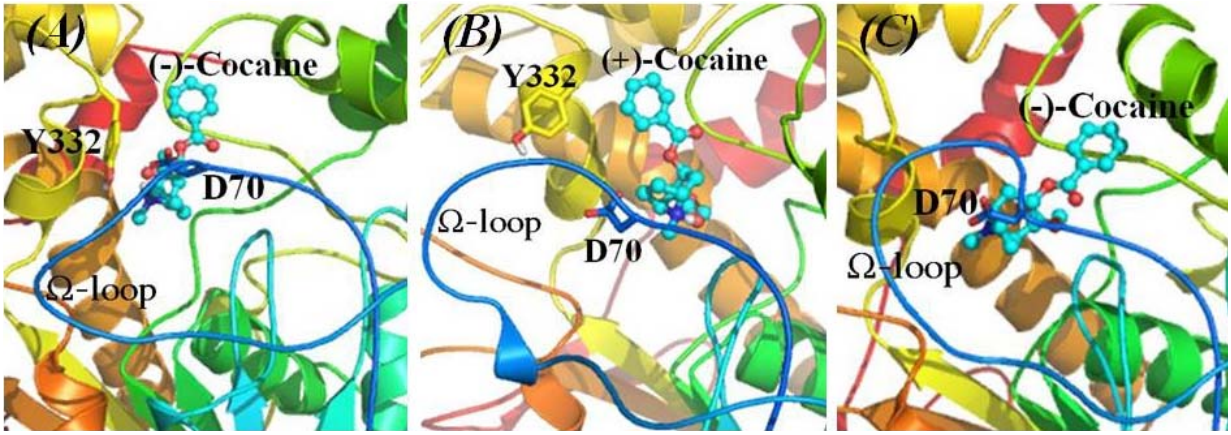

Figure S2. Typical ES1-like complex structure corresponding to the local free-energy minimum (*i.e*. a local minimum on the free energy surface) at the reaction coordinate value of ~16 to 17 Å for each of the enzyme-substrate binding complex systems. (A) wild-type BChE-(-)-cocaine binding complex derived from the snapshot at 109 ps of the MD trajectory in window 11 with the reaction coordinate value of 16.392 Å; (B) wild-type BChE-(+)-cocaine binding complex derived from the snapshot at 727 ps of the MD trajectory in window 13 with reaction coordinate value of 16.982 Å; and (C) A328W/Y332G mutant-(-)-cocaine binding complex derived from the snapshot at 619 ps of the MD trajectory in window 13 with the reaction coordinate value of 16.860 Å. BChE is represented as the colored ribbon, while (-)/(+)-cocaine molecule is shown as ball-and-stick. Residues D70 and/or Y332 are shown in stick style, and the Ω-loop is colored blue and labeled.

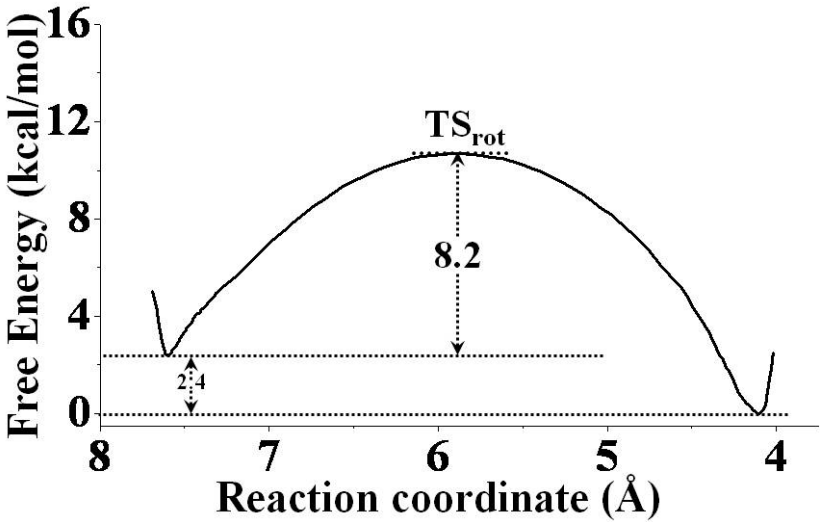

Figure S3. Free energy profile determined for the structural transformation of binding structure of wild-type BChE with (+)-cocaine. The reaction coordinate was defined as the distance from the mass center of benzoyl group of (+)-cocaine to the mass center of the side chain of S198 of wild-type BChE. The free energy barrier corresponding to TS_{rot} of the transformation process is also labeled (unit in kcal/mol). The potential of mean force (PMF) simulations were performed by using the same protocol as that in our previous study on the structural transformations for the binding structures of (-)-cocaine with both wild-type BChE and A328W/Y332G mutant (Huang, X.; Pan, Y.; Zheng, F.; Zhan, C.-G. *J. Phys. Chem. B* **2010**, *114*, 13545-13554).

Table S1. The tracked root-mean square deviation (RMSD) of the positions for the backbone atoms of the **Ω-loop** (residue #65 to #92) in the PMF-simulated **wild-type BChE-(+)-cocaine** complex from those in the **crystal structure** (PDB entry code 1P0P; RC represents the reaction coordinate for each PMF window; $RMSD_{min}$ is the smallest RMSD tracked from 1.0 ns MD trajectory for each window; $RMSD_{max}$ is the largest RMSD; $RMSD_{aver}$ is the averaged RMSD value; and SD refers to the standard deviation)

RC (Å)	$RMSD_{min}$ (Å)	$RMSD_{max}$ (Å)	$RMSD_{aver}$ (SD, Å)
10.24	0.80	2.61	1.43(0.32)
10.74	0.79	2.72	1.57 (0.42)
11.24	1.32	3.40	2.09 (0.31)
11.74	1.91	3.84	2.96 (0.29)
12.24	1.68	3.35	2.63 (0.27)
12.74	0.97	2.44	1.56 (0.21)
13.24	1.06	2.24	1.57 (0.16)
13.74	1.00	2.69	1.74 (0.25)
14.24	0.98	3.00	1.72 (0.39)
14.74	1.43	3.14	2.25 (0.22)
15.24	1.41	2.82	1.97 (0.20)
15.74	1.53	3.16	2.16 (0.30)
16.24	2.10	3.30	2.65 (0.20)
16.74	2.38	3.51	2.93 (0.19)
17.24	1.74	3.39	2.43 (0.34)
17.74	2.38	2.97	2.66 (0.10)
18.24	2.12	3.39	2.73 (0.23)
18.74	2.26	5.13	3.48 (0.50)
19.24	3.43	6.77	4.85 (0.73)
19.74	4.00	8.68	6.25 (1.12)
20.24	4.61	8.36	6.61 (0.58)
20.74	6.40	9.09	7.52 (0.46)

Table S2. The tracked root-mean square deviation (RMSD) of the positions for the backbone atoms of the **Ω-loop** (residue #65 to #92) in the PMF-simulated **wild-type BChE-(+)-cocaine** complex from those in the **starting structure** (RC represents the reaction coordinate for each PMF window; $RMSD_{min}$ is the smallest RMSD tracked from 1.0 ns MD trajectory for each window; $RMSD_{max}$ is the largest RMSD; $RMSD_{aver}$ is the averaged RMSD value; and SD refers to the standard deviation)

RC (Å)	$RMSD_{min}$ (Å)	$RMSD_{max}$ (Å)	$RMSD_{aver}$ (SD, Å)
10.24	0.30	2.01	1.41(0.22)
10.74	0.22	2.97	1.54 (0.65)
11.24	1.38	3.79	2.37 (0.40)
11.74	2.04	4.13	3.30 (0.31)
12.24	1.79	3.70	2.80 (0.33)
12.74	0.92	2.68	1.63 (0.26)
13.24	0.93	2.34	1.56 (0.20)
13.74	1.00	2.82	1.73 (0.29)
14.24	0.93	3.28	1.84 (0.52)
14.74	1.56	3.37	2.34 (0.27)
15.24	1.08	2.93	1.85 (0.30)
15.74	1.16	2.97	1.92 (0.37)
16.24	1.73	2.93	2.32 (0.21)
16.74	2.01	3.09	2.54 (0.18)
17.24	1.60	3.01	2.14 (0.27)
17.74	2.08	2.72	2.34 (0.10)
18.24	2.09	3.55	2.76 (0.30)
18.74	2.43	4.96	3.39 (0.40)
19.24	3.23	6.78	4.77 (0.76)
19.74	3.74	8.58	6.20 (1.14)
20.24	4.42	8.37	6.52 (0.67)
20.74	6.46	9.05	7.51 (0.46)

Table S3. For the PMF simulations on the **wild-type BChE-(-)-cocaine** binding, the tracked distance is between the cationic head (N^+) of (-)-cocaine and the negatively charged atom (OD1 or OD2) on the side chain of residue D70 of BChE. (RC represents the reaction coordinate for each PMF window; D_{min} is the shortest distance tracked from 1.0 ns MD trajectory for each window; D_{max} is the longest distance; D_{aver} is the averaged distance; and SD refers to the standard deviation)

RC (Å)	D_{min} (Å)	D_{max} (Å)	D_{aver} (SD, Å)
10.24	9.24	13.60	11.26 (0.56)
10.74	8.51	13.92	11.62 (0.67)
11.24	8.24	12.97	10.70 (0.89)
11.74	8.21	14.09	11.14 (0.86)
12.24	6.75	12.92	9.65 (1.12)
12.74	6.86	12.09	8.93 (0.86)
13.24	5.81	11.15	8.25 (1.04)
13.74	5.43	9.82	7.18 (0.79)
14.24	3.67	8.15	6.07 (0.74)
14.74	3.45	7.13	5.29 (0.57)
15.24	3.92	11.70	6.75 (1.33)
15.74	5.46	11.44	8.59 (1.00)
16.24	5.10	14.16	9.42 (1.49)
16.74	7.48	15.74	10.93 (1.48)
17.24	5.65	11.68	8.77 (0.96)
17.74	5.12	11.61	7.82 (0.93)
18.24	3.93	9.55	6.83 (1.03)
18.74	3.26	8.44	5.28 (0.93)
19.24	3.55	9.77	6.11 (1.28)
19.74	3.27	9.33	5.94 (1.21)
20.24	2.91	8.58	4.47 (0.99)
20.74	3.49	11.29	6.63 (1.46)

Table S4. For the PMF simulations on the **wild-type BChE-(+)-cocaine** binding, the tracked distance is between the cationic head (N^+) of (+)-cocaine and the negatively charged atom (OD1 or OD2) on the side chain of residue D70 of BChE. (RC represents the reaction coordinate for each PMF window; D_{min} is the shortest distance tracked from 1.0 ns MD trajectory for each window; D_{max} is the longest distance; D_{aver} is the averaged distance; and SD refers to the standard deviation)

RC (Å)	D_{min} (Å)	D_{max} (Å)	D_{aver} (SD, Å)
10.24	7.24	15.38	11.15 (0.88)
10.74	7.64	15.82	11.77 (1.68)
11.24	9.90	15.69	12.78 (0.94)
11.74	11.58	18.10	14.49 (0.93)
12.24	9.63	15.62	12.79 (0.96)
12.74	5.00	13.21	8.99 (1.31)
13.24	4.08	10.81	6.50 (0.92)
13.74	3.52	10.86	6.21 (1.14)
14.24	3.83	7.72	5.83 (0.63)
14.74	7.67	11.68	10.03 (0.70)
15.24	5.01	12.38	8.58 (1.16)
15.74	4.56	12.48	8.89 (1.44)
16.24	6.89	10.92	8.95 (0.54)
16.74	6.01	10.97	8.66 (0.65)
17.24	4.64	10.83	7.54 (1.14)
17.74	5.21	11.16	7.64 (0.91)
18.24	5.03	12.85	9.07 (1.20)
18.74	5.61	12.88	8.65 (1.08)
19.24	7.49	16.39	11.85 (1.87)
19.74	7.11	17.53	13.72 (1.86)
20.24	6.12	16.25	10.65 (1.86)
20.74	11.19	17.09	14.25 (0.94)

Table S5. For the PMF simulations on the **A328W/Y332G mutant-(-)-cocaine** binding, the tracked distance is between the cationic head (N^+) of (+)-cocaine and the negatively charged atom (OD1 or OD2) on the side chain of residue D70 of BChE. (RC represents the reaction coordinate for each PMF window; D_{min} is the shortest distance tracked from 1.0 ns MD trajectory for each window; D_{max} is the longest distance; D_{aver} is the averaged distance; and SD refers to the standard deviation)

RC (Å)	D_{min} (Å)	D_{max} (Å)	D_{aver} (SD, Å)
10.35	8.39	13.83	11.37 (1.02)
10.85	9.07	17.64	12.85 (1.56)
11.35	10.30	17.40	13.45 (1.19)
11.85	12.29	18.98	16.68 (0.74)
12.35	11.66	18.53	15.94 (1.13)
12.85	8.70	17.23	11.90 (1.40)
13.35	10.28	17.93	14.94 (1.12)
13.85	11.78	16.12	13.79 (0.65)
14.35	9.31	15.89	13.04 (0.94)
14.85	11.56	16.91	14.28 (0.89)
15.35	9.40	14.79	12.29 (0.86)
15.85	8.99	14.82	11.55 (0.93)
16.35	9.10	13.68	10.87 (0.66)
16.85	8.56	14.45	11.13 (0.66)
17.35	8.86	13.97	11.05 (0.71)
17.85	9.39	15.24	12.39 (0.67)
18.35	9.87	14.60	11.97 (0.76)
18.85	9.77	16.01	12.84 (1.17)
19.35	9.22	16.42	12.95 (1.15)
19.85	9.89	16.32	13.31 (0.87)
20.35	11.84	16.77	14.52 (0.68)
20.85	10.97	17.03	13.65 (1.13)