Supporting Information

Reaction Pathway and Free Energy Profile for Pre-Chemical Reaction Step of Human Butyrylcholinesterase-Catalyzed Hydrolysis of (-)-Cocaine by Combined Targeted Molecular Dynamics and Potential of Mean Force Simulations

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Running title: Pre-chemical reaction step of (-)-cocaine in BChE

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Supporting Information Available. Two figures for the results of MD simulations on the binding structures of (-)-cocaine binding with both wild-type BChE and A328W/Y332G mutant; one figure for the tracked residue-based contacts of A328W/Y332G BChE with (-)-cocaine along the reaction coordinate; two tables about the number and fraction of calculated residue-based contacts between (-)-cocaine and BChE. This material is available free of charge via the Internet http://pubs.acs.org.

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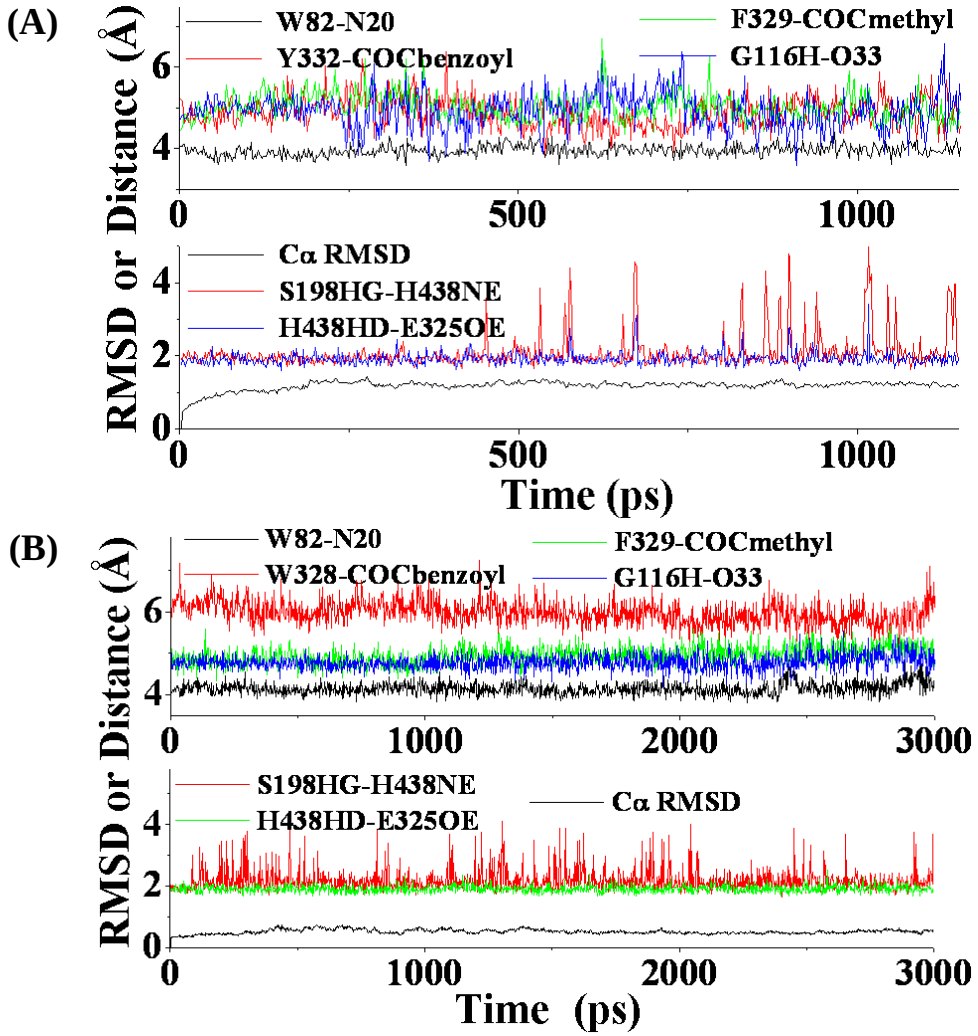

Figure S1. (A) Plots of Cα RMSDs and key distances tracked through MD simulation on the nonprereactive complex for wild-type BChE-(-)-cocaine binding. W82-N20 represents the distance from the center of aromatic side chain of W82 to the nitrogen atom at the cationic head of (-)-cocaine; Y332-COCbenzoyl means the distance from the center of aromatic side chain of Y332 to the center of benzoyl group of (-)-cocaine; F329-COCmethyl represents the distance from the center of aromatic side chain of F329 to the center of methyl ester group of (-)-cocaine; G116H-O33 represents the distance between the backbone hydrogen of residue G116 to the carbonyl oxygen at the benzoyl ester of (-)-cocaine, and similar meaning for distance as G117H-O33; S198HG-H438NE and H438HD-E325OE represent the hydrogen bonding distances within the catalytic triad residues S198-H438-E325 of the enzyme. (B) Tracked changes for Cα RMSDs and key

distances from MD simulation on the nonprereactive complex for A328W/Y332G BChE-(-)-cocaine binding. Similarly, W328-COCbenzoyl means the distance from the center of aromatic side chain of W328 to the center of benzoyl group of (-)-cocaine.

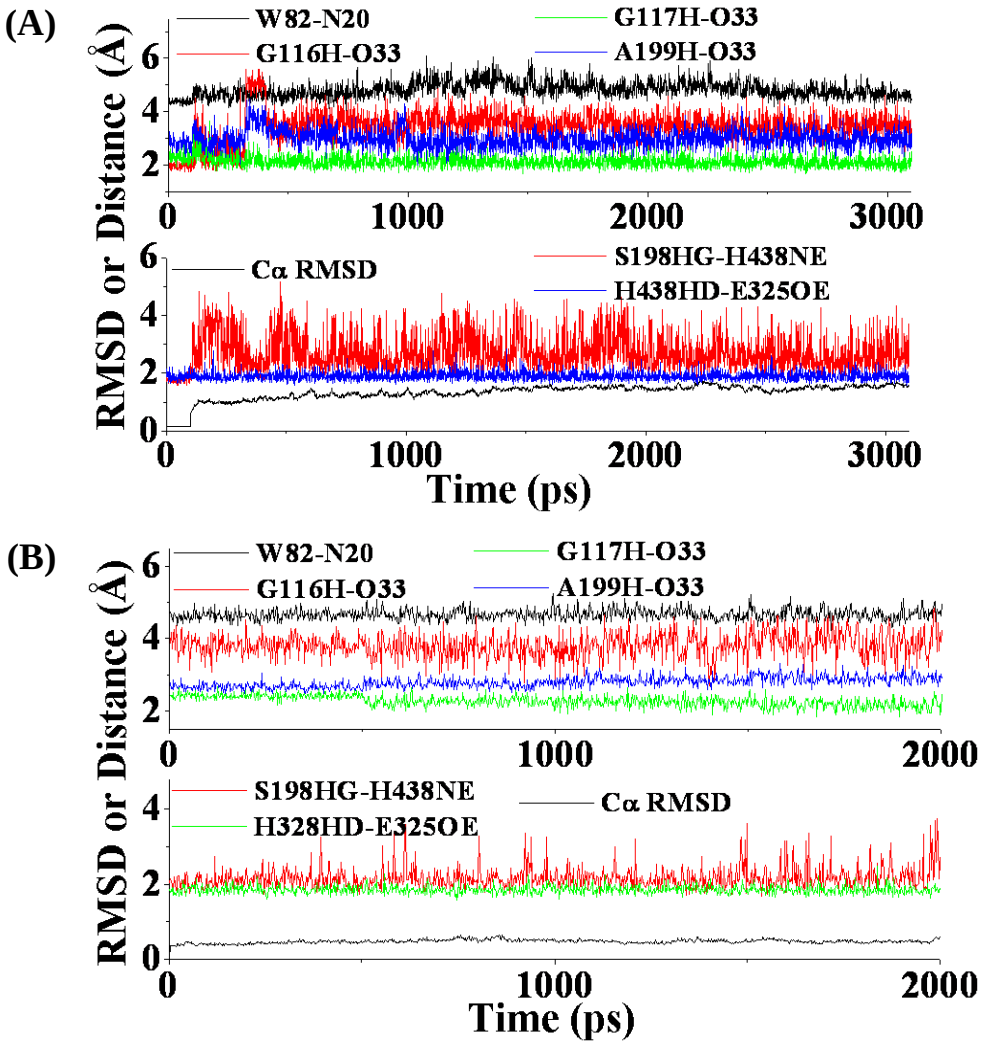

Figure S2. Plots of Cα RMSDs and key distances tracked through MD simulation on the prereactive complex for wild-type BChE-(-)-cocaine binding structure (A) and A328W/Y332G BChE-(-)-cocaine binding structure (B). W82-N20 represents the distance from the center of aromatic side chain of W82 to the nitrogen atom at the cationic head of (-)-cocaine; G116H-O33 represents the distance between the backbone hydrogen of residue G116 to the carbonyl oxygen at the benzoyl ester of (-)-cocaine, and similar meaning for distances as G117H-O33 and A199H-O33, respectively; S198HG-

H438NE and H438HD-E325OE represent the hydrogen bonding distances within the catalytic triad residues S198-H438-E325 of the enzyme.

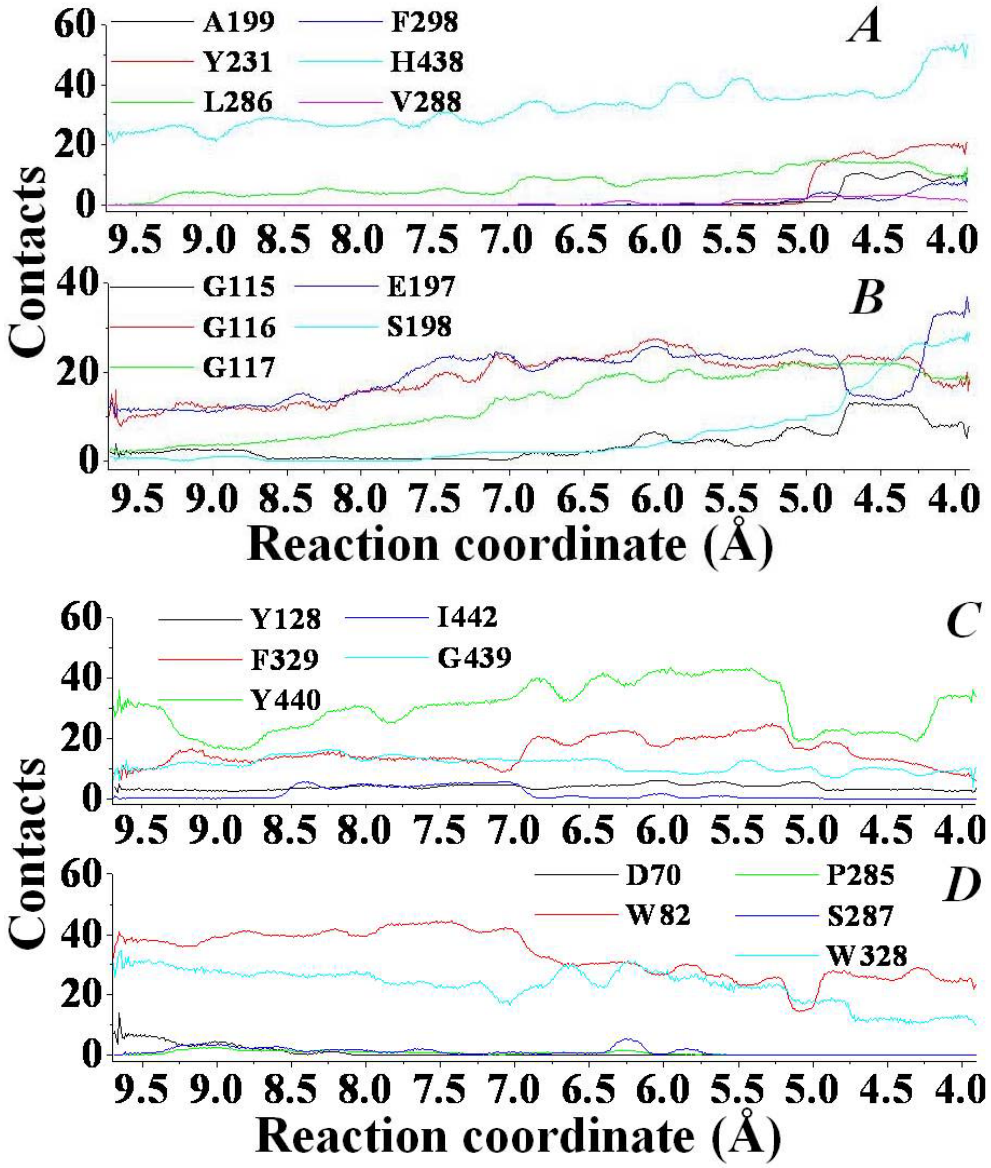

Figure S3. Plots for residue-based contacts of A328W/Y332G mutant interacting with (-)-cocaine along the reaction coordinate (*i.e.* the distance from the mass center of the benzoyl group of (-)-cocaine to the mass center of the side chain of residue S198 of the enzyme). (A) and (B): Type I residues showing significant increase in the number of contacts. (C) Type II residues with continuous contacts, and (D) type III residues with decreased number of contacts.

Table S1. Residues of Wild-type BChE Having Contacts with (-)-Cocaine along the TMD Simulations (Distance Cutoff=5.0Å, Fraction Cutoff=0.080)

Residue	Contacts[a]	Fraction[b]	Features*
W82	30.73	1.00	C decreased
G116	20.73	1.00	C increased
G117	15.08	1.00	C increased
E197	21.29	1.00	C increased
L286	10.03	1.00	C to C
F329	24.24	1.00	C to C
H438	34.59	1.00	C increased
Y440	30.25	1.00	C to C
G439	7.96	1.00	C to C
S198	7.46	0.98	C increased
Y128	2.61	0.97	C to C
G115	4.26	0.90	C increased
A328	5.03	0.82	C decreased
V288	1.91	0.64	C increased
A199	0.96	0.46	C increased
Y332	6.39	0.45	C decreased
F398	2.34	0.42	C increased
W231	5.91	0.41	C increased
P285	1.25	0.41	C decreased
S287	1.81	0.41	C decreased
T120	0.34	0.21	C decreased
I442	0.53	0.19	C to C
D70	0.62	0.18	C decreased
S224	0.27	0.16	C increased
M437	0.52	0.13	C to C

[a] Average number of contacts over the entire TMD trajectory. [b] Fraction of time in which at least one contact was formed during the TMD simulation. * C: contacted; C to C: contacted continuously.

Table S2. Residues of the A328W/Y332G mutant having contacts with (-)-Cocaine during the TMD simulations (distance cutoff=5.0 Å, fraction cutoff=0.080)

Residue	Contacts[a]	Fraction[b]	Features*
W82	30.02	1.00	C decreased
G116	18.74	1.00	C increased
G117	14.60	0.98	C increased
Y128	3.62	0.99	C to C
E197	19.87	1.00	C increased
L286	8.40	0.99	C increased
W328	22.51	1.00	C decreased
F329	17.59	1.00	C to C
H438	34.62	1.00	C increased
G439	10.47	1.00	C to C
Y440	28.32	0.99	C to C
G115	3.92	0.79	C increased
S198	6.27	0.78	C increased
V288	0.87	0.42	C increased
I442	1.44	0.41	C to C
P285	0.70	0.39	C decreased
A199	1.24	0.37	C increased
S287	1.14	0.31	C decreased
F398	1.03	0.28	C increased
D70	1.11	0.20	C decreased
W231	2.63	0.18	C increased

[a] Average number of contacts over the entire TMD trajectory. [b] Fraction of time in which at least one contact was formed during the TMD simulation. * C: contacted; C to C: contacted continuously.