Supporting Information

Free-Energy Perturbation Simulation on Transition States and High-Activity Mutants of Human Butyrylcholinesterase for (-)-Cocaine Hydrolysis

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Tleap input file for setting TS1 Molecular Dynamic Simulation

```
source leaprc.ff03
source leaprc.gaff

loadamberprep L4_TS1.prep
loadamberparams COG_TS1-QMMM-half-force.frcmod
x = loadpdb pdb

#   ============================================================
#     Disulfide Bond Settings
#   ============================================================
bond x.65.SG x.92.SG
bond x.252.SG x.263.SG
bond x.400.SG x.519.SG

#   ============================================================
#     TS1 Geometrical Restraint Settings
#   ============================================================
set x.533.C32 element C

set x.198.HG type GO
set x.198.HG pertType GO
set x.198.HG pert true

bond x.198.OG x.533.C32
bond x.198.HG x.438.NE2

#   ============================================================
#     TS1 RESP Charge Settings
#   ============================================================
set x.198.CB       charge     0.943593
set x.198.HB2    charge    -0.342638
set x.198.HB3    charge    -0.342638
set x.198.OG       charge    -0.768909
set x.198.HG       charge     0.270328
set x.325.CG       charge     0.373161
set x.325.HG2    charge    -0.147799
set x.325.HG3    charge    -0.147799
set x.325.CD       charge     0.772258
set x.325.OE1     charge    -0.815823
set x.325.OE2     charge    -0.815823
set x.438.CB        charge     0.773533
set x.438.HB2     charge    -0.241844
```

```
set x.438.HB3      charge    -0.241844
set x.438.CG         charge    -0.027996
set x.438.ND1    charge    -0.130300
set x.438.HD1    charge     0.133114
set x.438.CE1    charge    -0.000184
set x.438.HE1    charge     0.273829
set x.438.NE2    charge    -0.075426
set x.438.CD2    charge    -0.296473
set x.438.HD2    charge     0.223556
set x.533.C1     charge    -0.080305
set x.533.H2     charge     0.119641
set x.533.H3     charge     0.119641
set x.533.H4     charge     0.119641
set x.533.O5     charge    -0.435106
set x.533.C6     charge     0.989364
set x.533.O7     charge    -0.692263
set x.533.C8     charge    -0.584711
set x.533.H9     charge     0.286167
set x.533.C10    charge    -0.107359
set x.533.N20    charge    -0.472431
set x.533.C22    charge    -0.881557
set x.533.H23    charge     0.333947
set x.533.H24    charge     0.333947
set x.533.H25    charge     0.333947
set x.533.H21    charge     0.553621
set x.533.H11    charge     0.222974
set x.533.C12    charge     0.197362
set x.533.H13    charge     0.041417
set x.533.H14    charge     0.041417
set x.533.C15    charge    -0.378847
set x.533.H16    charge     0.123321
set x.533.H17    charge     0.123321
set x.533.C18    charge     0.583020
set x.533.H19    charge     0.047197
set x.533.C26    charge    -0.995627
set x.533.H27    charge     0.239247
set x.533.H28    charge     0.239247
set x.533.C29    charge     1.082902
set x.533.H30    charge    -0.043165
set x.533.O31    charge    -0.529473
set x.533.C32    charge     0.223308
set x.533.O33    charge    -0.634238
set x.533.C34    charge     0.285634
set x.533.C35    charge    -0.188575
```

```
set x.533.H36    charge      0.134039
set x.533.C37    charge    -0.167364
set x.533.H38    charge      0.162738
set x.533.C39    charge    -0.153713
set x.533.H40    charge      0.189759
set x.533.C41    charge    -0.167364
set x.533.H42    charge      0.162739
set x.533.C43    charge    -0.188575
set x.533.H44    charge      0.134039

#    -- For backbone atoms of QM residues --
#    -- taken from AMBER ff03 (all_amino03.in)

set x.198.N           charge    -0.541400
set x.198.H           charge      0.345400
set x.198.CA      charge      0.118100
set x.198.HA      charge      0.142200
set x.198.C           charge      0.483400
set x.198.O           charge    -0.580800
set x.325.N           charge    -0.423400
set x.325.H           charge      0.306800
set x.325.CA      charge      0.031600
set x.325.HA      charge      0.065100
set x.325.CB      charge      0.074800
set x.325.HB2 charge    -0.003500
set x.325.HB3 charge    -0.003500
set x.325.C           charge      0.469700
set x.325.O           charge    -0.592500
set x.438.N           charge    -0.506800
set x.438.H           charge      0.351000
set x.438.CA      charge      0.119100
set x.438.HA      charge      0.137800
set x.438.C           charge      0.515900
set x.438.O           charge    -0.599800

#   ============================================================
#     Save Topology files
#   ============================================================
addions x Cl- 0
solvatebox x TIP3PBOX 10
saveAmberParm x x.top x.crd
quit
```

The content of *COG_TS1-QMMM-half-force.frcmod* used in the tleap input file

```
MASS
cx      12.01               0.616               Sp2 C carbonyl group |      Carboxyl carbon (C32)
GN 1.008                0.161               Hydrogen on N       |      GN is the hydrogen between
His and Glu (HD1)
GO 1.008                0.135               Hydroxyl group H          |      Ser198 hydroxyl
hydrogen (HG or HE2)

BOND
cx-os           200.9       1.420           COG in TS1
cx-o             270.2       1.240           COG in TS1
cx-OH       234.1       2.030           COG in TS1              | OH is the atom type of Ser198
hydroxyl oxygen
cx-ca           173.2       1.500           COG in TS1
GN-NA    217.0       1.070           HD1 in HIS of TS1
GN-O2     217.0       1.600           Added bond for TS1
GO-OH    276.5       1.520           HG in SER of TS1
GO-NB    276.5       1.110           added bond

ANGLE
os-c -ca                67.662      110.765     Calculated with empirical approach
cx-os-c3                63.0            120.79          NEW_MP2/6-31G COG
o -cx-os                75.4            118.38          MP2/6-31G* COG
os-cx-ca                67.662      112.01          same as os-c -ca
ca-cx-o                 67.1            122.55          NEW_MP2/6-31G COG
cx-ca-ca                62.0            121.66          NEW_MP2/6-31G COG
CT-OH-cx        50.5            107.38          new type for COG and ser197
OH-cx-os          60.5            98.38           new type reduced force
OH-cx-o           60.5            106.26          only bond length important
ca-cx-OH          50.9            91.41
cx-OH-GO       50.9            117.88          above are for COG
CC-NA-GN    50.0            131.50          GN is H in his in TS1           |    CC is atom
type of CG
CR-NA-GN    50.0            120.22          GN is H, his in TS1             |    CR is atom
type of CE1
CT-OH-GO     55.0            104.59          GO is HG in SER for TS1
C -O2-GN         50.1            135.41
```

O2-GN-NA	50.1	155.46	
OH-GO-NB	50.1	157.82	
GO-NB-CR	50.1	132.64	
GO-NB-CV	50.1	117.98	

DIHE

X -cx-os-X	2	5.400	180.000	2.000
X -cx-ca-X	4	14.500	180.000	2.000 intrpol.bsd.on C6H6
X -cx-OH-X	2	0.000	0.000	3.000 3-fold, no force
CT-C -O2-GN	2	0.000	180.000	2.000
C -O2-GN-NA	2	0.000	180.000	2.000
O2-C -O2-GN	2	0.000	180.000	2.000
O2-GN-NA-CC	2	0.000	180.000	2.000
O2-GN-NA-CR	2	0.000	180.000	2.000
CT-OH-GO-NB	2	0.000	180.000	2.000
OH-GO-NB-CR	2	0.000	180.000	2.000
OH-GO-NB-CV	2	0.000	180.000	2.000
cx-OH-GO-NB	2	0.000	180.000	2.000

IMPROPER

c3-o -c -os	1.1	180.0	2.0	Using default value
ca-o -c -os	1.1	180.0	2.0	Using default value
c -ca-ca-ca	1.1	180.0	2.0	Using default value
ca-ca-ca-ha	1.1	180.0	2.0	Using default value
c3-o -cx-os	1.1	180.0	2.0	Using default value
ca-o -cx-os	1.1	180.0	2.0	Using default value
cx-ca-ca-ca	1.1	180.0	2.0	Using default value

NONBON

cx	1.9080	0.0860	OPLS (c type)
GN	0.6000	0.0157	Hydrogen on N
GO	0.0000	0.0000	Hydroxyl H

Table S1. The ΔG_E or ΔG_{TS1} values (in kcal/mol) obtained from the FEP simulations using five different initial structures associated with different snapshots of the MD trajectory.

Mutation simulated	Energy change	FEP direction	Calculated ΔG_E or ΔG_{TS1} values using various initial structures					Average value
A199S/A328W/Y332G ↓ A199S/F227A/A328W/Y332G	ΔG_E	Forward	5.76	5.98	6.58	6.23	6.26	6.16
		Backward	-5.94	-6.03	-6.65	-6.31	-5.45	-6.08
	ΔG_{TS1}	Forward	5.11	5.12	5.24	4.73	4.55	4.95
		Backward	-5.06	-5.20	-5.32	-4.89	-4.63	-5.02
A199S/A328W/Y332G ↓ A199S/S287A/A328W/Y332G	ΔG_E	Forward	8.32	8.75	8.67	8.37	8.57	8.54
		Backward	-8.43	-8.84	-8.74	-8.47	-8.65	-8.63
	ΔG_{TS1}	Forward	6.98	7.34	7.98	8.09	8.32	7.74
		Backward	-7.07	-7.39	-8.04	-8.20	-8.40	-7.82
A199S/S287A/A328W/Y332G ↓ A199S/S287G/A328W/Y332G	ΔG_E	Forward	-6.29	-6.45	-5.46	-4.92	-5.63	-5.75
		Backward	6.03	6.28	5.28	4.78	5.55	5.58
	ΔG_{TS1}	Forward	-6.62	-5.07	-6.20	-5.75	-5.83	-5.89
		Backward	6.46	4.89	6.06	5.61	5.67	5.74
A199S/S287G/A328W/Y332G ↓ A199S/F227A/S287G/A328W/Y332G	ΔG_E	Forward	6.76	5.95	6.81	7.22	7.15	6.78
		Backward	-6.94	-6.10	-6.94	-7.32	-7.24	-6.91
	ΔG_{TS1}	Forward	5.78	4.95	5.48	6.51	5.84	5.71
		Backward	-5.90	-5.15	-5.67	-6.71	-5.97	-5.88