Supplementary Information

Substrate Selectivity of High-Activity Mutants of Human Butyrylcholinesterase

Shurong Hou,[1] Liu Xue,[1] Wenchao Yang,[1] Lei Fang, Fang Zheng, and Chang-Guo Zhan

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536

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Correspondence:

Chang-Guo Zhan, Ph.D.

Professor

Department of Pharmaceutical Sciences

College of Pharmacy

University of Kentucky

789 South Limestone Street

Lexington, KY 40536

TEL: 859-323-3943

E-mail: zhan@uky.edu

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[1] These authors contributed equally to the work.

Table S1. Summary of the MD-simulated key distances (in Å) and the calculated total hydrogen-bonding energies (tHBE, in kcal/mol) between the oxyanion hole and the carbonyl oxygen of (-)-cocaine in the ES and TS1 structures.

Complex Structures			Distance[a]				tHBE[b]
			D1	D2	D3	D4	
Wild-type BChE	ES	Average	3.35	2.02	3.45		-3.73 (-3.04)
		Maximum	4.50	2.70	4.41		
		Minimum	2.24	1.63	2.45		
		Fluctuation	0.33	0.16	0.26		
		HBE	-0.047 (-0.028)	-3.65 (-2.99)	-0.027 (-0.019)		
	TS1	Average	3.38	2.14	2.09		-5.14 (-4.12)
		Maximum	4.33	3.12	2.71		
		Minimum	3.11	1.68	1.70		
		Fluctuation	0.12	0.18	0.15		
		HBE	-0.028 (-0.026)	-2.35 (-1.82)	-2.75 (-2.27)		
E12-7[c]	ES	Average	3.00	2.12	3.16	2.57	-3.18 (-2.42)
		Maximum	4.00	2.69	3.88	3.90	
		Minimum	2.25	1.69	2.58	1.89	
		Fluctuation	0.25	0.16	0.20	0.36	
		HBE	-0.11 (-0.079)	-2.39 (-1.96)	-0.06 (-0.049)	-0.61 (-0.34)	
	TS1	Average	2.65	2.18	2.00	1.72	-18.14 (-15.58)
		Maximum	4.62	3.18	2.65	2.19	
		Minimum	1.65	1.71	1.65	1.47	
		Fluctuation	0.57	0.14	0.19	0.10	
		HBE	-11.32 (-10.58)	-2.07 (-1.56)	-3.67 (-3.14)	-1.08 (-0.26)	

[a] D1, D2, and D3 refer to the distances between the carbonyl oxygen of (-)-cocaine and the NH hydrogen of G116, G117, and A199 (in wild-type BChE)/S199 (in E12-7), respectively. D4 is the distance between the carbonyl oxygen of (-)-cocaine and the hydroxyl hydrogen of the S199 side chain in E12-7.

[b] The total HBE value is the average of the HBE values calculated by using the instantaneous distances in all of the snapshots. The values given in the parentheses were calculated by using the MD-simulated average distances. Based on the general HBE equation, we have $HBE(r) \approx 5\varepsilon r_0^{12}/r^{12} - 6\varepsilon r_0^{10}/r^{10}$, in which r is the H···O distance in the considered hydrogen bond and r_0 (= 1.45 Å) is the minimum value of the H···O distance for which the HBE equation can be used. The ε value was determined by using the condition that $HBE(r) = -5.0$ kcal/mol when $r = 1.90$ Å.

[c] E12-7 is the A199S/F227A/S287G/A328W/Y332G mutant of human BChE.

Table S2. Summary of the MD-simulated key distances (in Å) and the calculated total hydrogen-bonding energies (tHBE, in kcal/mol) between the oxyanion hole and the carbonyl oxygen of (+)-cocaine in the ES and TS1 structures.

Complex Structures		Distance[a]					tHBE[b]
			D1	D2	D3	D4	
Wild-type BChE	ES	Average	3.08	2.48	2.04		-4.47 (-3.32)
		Maximum	4.10	3.49	3.25		
		Minimum	2.25	1.80	1.64		
		Fluctuation	0.29	0.28	0.20		
		HBE	-0.09 (-0.06)	-0.82 (-0.49)	-3.55 (-2.77)		
	TS1	Average	1.90	2.00	2.14		-11.89 (-9.90)
		Maximum	3.91	2.56	2.68		
		Minimum	1.61	1.65	1.74		
		Fluctuation	0.20	0.14	0.15		
		HBE	-5.97 (-4.89)	-3.80 (-3.24)	-2.12 (-1.76)		
E12-7[c]	ES	Average	3.50	2.38	2.50	4.18	-1.60 (-1.19)
		Maximum	3.96	3.07	3.55	4.97	
		Minimum	3.07	1.85	1.84	3.42	
		Fluctuation	0.14	0.18	0.24	0.24	
		HBE	-0.020 (-0.018)	-0.90 (-0.71)	-0.68 (-0.46)	0.0 (0.0)	
	TS1	Average	1.90	2.04	2.01	3.57	-13.54 (-10.92)
		Maximum	4.29	2.61	2.62	4.48	
		Minimum	1.50	1.62	1.67	2.42	
		Fluctuation	0.22	0.14	0.14	0.30	
		HBE	-6.58 (-5.04)	-3.67 (-3.09)	-3.26 (-2.78)	-0.022 (-0.014)	

[a] D1, D2, and D3 refer to the distances between the carbonyl oxygen of (+)-cocaine and the NH hydrogen of G116, G117, and A199 (in wild-type BChE)/S199 (in E12-7), respectively. D4 is the distance between the carbonyl oxygen of (+)-cocaine and the hydroxyl hydrogen of the S199 side chain in E12-7.

[b] The total HBE value is the average of the HBE values calculated by using the instantaneous distances in all of the snapshots. The values given in the parentheses were calculated by using the MD-simulated average distances. Based on the general HBE equation, we have $HBE(r) \approx 5\varepsilon r_0^{12}/r^{12} - 6\varepsilon r_0^{10}/r^{10}$, in which r is the H···O distance in the considered hydrogen bond and r_0 (= 1.45 Å) is the minimum value of the H···O distance for which the HBE equation can be used. The ε value was determined by using the condition that $HBE(r) = -5.0$ kcal/mol when $r = 1.90$ Å.

[c] E12-7 is the A199S/F227A/S287G/A328W/Y332G mutant of human BChE.

Table S3. Summary of the MD-simulated key distances (in Å) and the calculated total hydrogen-bonding energies (tHBE, in kcal/mol) between the oxyanion hole and the carbonyl oxygen of ACh in the ES and TS1 structures.

Complex Structures		Distance[a]					tHBE[b]
			D1	D2	D3	D4	
Wild-type BChE	ES	Average	2.42	2.33	3.48		-2.08 (-1.49)
		Maximum	3.36	3.01	4.10		
		Minimum	1.81	1.81	2.83		
		Fluctuation	0.25	0.18	0.19		
		HBE	-0.95 (-0.60)	-1.11 (-0.87)	-0.03 (-0.03)		
	TS1	Average	1.94	1.87	1.99		-15.11 (-13.13)
		Maximum	2.99	2.31	2.50		
		Minimum	1.59	1.61	1.64		
		Fluctuation	0.17	0.11	0.12		
		HBE	-5.04 (-4.13)	-6.16 (-5.58)	-3.90 (-3.42)		
E12-7[c]	ES	Average	2.26	2.32	3.54	3.41	-2.77 (-2.06)
		Maximum	3.44	3.06	4.21	4.13	
		Minimum	1.68	1.80	2.99	2.82	
		Fluctuation	0.20	0.19	0.17	0.19	
		HBE	-1.54 (-1.12)	-1.18 (-0.90)	-0.024 (-0.016)	-0.028 (-0.024)	
	TS1	Average	1.76	1.97	2.12	2.82	-17.72 (-15.16)
		Maximum	2.78	2.55	2.81	4.15	
		Minimum	1.45	1.64	1.67	1.68	
		Fluctuation	0.13	0.16	0.14	0.47	
		HBE	-10.30 (-9.23)	-4.37 (-3.78)	-2.40 (-1.97)	-0.65 (-0.14)	

[a] D1, D2, and D3 refer to the distances between the carbonyl oxygen of ACh and the NH hydrogen of G116, G117, and A199 (in wild-type BChE)/S199 (in E12-7), respectively. D4 is the distance between the carbonyl oxygen of ACh and the hydroxyl hydrogen of the S199 side chain in E12-7.

[b] The total HBE value is the average of the HBE values calculated by using the instantaneous distances in all of the snapshots. The values given in the parentheses were calculated by using the MD-simulated average distances. Based on the general HBE equation, we have $HBE(r) \approx 5\varepsilon r_0^{12}/r^{12} - 6\varepsilon r_0^{10}/r^{10}$, in which r is the H···O distance in the considered hydrogen bond and r_0 (= 1.45 Å) is the minimum value of the H···O distance for which the HBE equation can be used. The ε value was determined by using the condition that $HBE(r) = -5.0$ kcal/mol when $r = 1.90$ Å.

[c] E12-7 is the A199S/F227A/S287G/A328W/Y332G mutant of human BChE.

Table S4. Summary of the MD-simulated key distances (in Å) and the calculated total hydrogen-bonding energies (tHBE, in kcal/mol) between the oxyanion hole and the carbonyl oxygen of ATC in the ES and TS1 structures.

Complex Structures		Distance[a]					tHBE[b]
			D1	D2	D3	D4	
Wild-type BChE	ES	Average	2.43	2.37	3.97		-1.95 (-1.32)
		Maximum	3.30	3.21	4.67		
		Minimum	1.74	1.79	3.41		
		Fluctuation	0.25	0.21	0.19		
		HBE	-0.92 (0.58)	-1.03 (0.74)	0.0 (0.0)		
	TS1	Average	1.93	1.85	1.96		-16.28 (-14.41)
		Maximum	2.91	2.36	2.58		
		Minimum	1.59	1.59	1.62		
		Fluctuation	0.15	0.10	0.13		
		HBE	-5.08 (-4.31)	-6.71 (-6.19)	-4.49 (-3.92)		
E12-7[c]	ES	Average	2.23	2.22	3.76	3.62	-3.39 (-2.59)
		Maximum	3.09	3.04	3.97	3.79	
		Minimum	1.74	1.80	3.23	2.97	
		Fluctuation	0.21	0.16	0.16	0.22	
		HBE	-1.73 (1.25)	-1.64 (1.32)	-0.01 (-0.01)	-0.016 (-0.013)	
	TS1	Average	1.66	1.96	2.0	2.76	-22.83 (20.83)
		Maximum	2.10	2.43	2.70	4.2	
		Minimum	1.45	1.66	1.66	1.66	
		Fluctuation	0.09	0.13	0.13	0.42	
		HBE	-14.0 (-13.53)	-4.39 (-3.82)	-3.83 (3.30)	-0.61 (-0.18)	

[a] D1, D2, and D3 refer to the distances between the carbonyl oxygen of ATC and the NH hydrogen of G116, G117, and A199 (in wild-type BChE)/S199 (in E12-7), respectively. D4 is the distance between the carbonyl oxygen of ATC and the hydroxyl hydrogen of the S199 side chain in E12-7.

[b] The total HBE value is the average of the HBE values calculated by using the instantaneous distances in all of the snapshots. The values given in the parentheses were calculated by using the MD-simulated average distances. Based on the general HBE equation, we have $HBE(r) \approx 5\varepsilon r_0^{12}/r^{12} - 6\varepsilon r_0^{10}/r^{10}$, in which r is the H···O distance in the considered hydrogen bond and r_0 (= 1.45 Å) is the minimum value of the H···O distance for which the HBE equation can be used. The ε value was determined by using the condition that $HBE(r) = -5.0$ kcal/mol when $r = 1.90$ Å.

[c] E12-7 is the A199S/F227A/S287G/A328W/Y332G mutant of human BChE.

Table S5. Summary of the MD-simulated key distances (in Å) and the calculated total hydrogen-bonding energies (tHBE, in kcal/mol) between the oxyanion hole and the carbonyl oxygen of BTC in the ES and TS1 structures.

Complex Structures		Distance[a]					tHBE[b]
			D1	D2	D3	D4	
Wild-type BChE	ES	Average	2.05	2.12	3.49		-5.52 (-4.68)
		Maximum	3.03	2.83	4.20		
		Minimum	1.67	1.73	2.64		
		Fluctuation	0.14	0.15	0.21		
		HBE	-3.12 (2.70)	-2.38 (-1.96)	-0.022 (-0.019)		
	TS1	Average	1.99	1.90	2.08		-12.76 (-10.82)
		Maximum	3.08	2.78	2.66		
		Minimum	1.63	1.59	1.64		
		Fluctuation	0.17	0.12	0.15		
		HBE	-4.16 (-3.34)	-5.71 (-5.09)	-2.89 (-2.38)		
E12-7[c]	ES	Average	2.25	2.31	3.47	3.63	-2.83 (-2.09)
		Maximum	3.07	2.94	4.19	4.90	
		Minimum	1.75	1.83	2.85	2.45	
		Fluctuation	0.22	0.18	0.18	0.35	
		HBE	-1.62 (-1.14)	-1.17 (-0.92)	-0.023 (-0.020)	-0.020 (-0.013)	
	TS1	Average	1.78	2.18	1.97	3.85	-16.04 (-13.68)
		Maximum	2.59	2.85	2.69	4.90	
		Minimum	1.47	1.66	1.64	1.85	
		Fluctuation	0.14	0.19	0.14	0.43	
		HBE	-9.62 (-8.42)	-2.08 (-1.55)	-4.30 (-3.70)	-0.032 (-0.007)	

[a] D1, D2, and D3 refer to the distances between the carbonyl oxygen of BTC and the NH hydrogen of G116, G117, and A199 (in wild-type BChE)/S199 (in E12-7), respectively. D4 is the distance between the carbonyl oxygen of BTC and the hydroxyl hydrogen of the S199 side chain in E12-7.

[b] The total HBE value is the average of the HBE values calculated by using the instantaneous distances in all of the snapshots. The values given in the parentheses were calculated by using the MD-simulated average distances. Based on the general HBE equation, we have $HBE(r) \approx 5\varepsilon r_0^{12}/r^{12} - 6\varepsilon r_0^{10}/r^{10}$, in which r is the H···O distance in the considered hydrogen bond and r_0 (= 1.45 Å) is the minimum value of the H···O distance for which the HBE equation can be used. The ε value was determined by using the condition that $HBE(r) = -5.0$ kcal/mol when $r = 1.90$ Å.

[c] E12-7 is the A199S/F227A/S287G/A328W/Y332G mutant of human BChE.

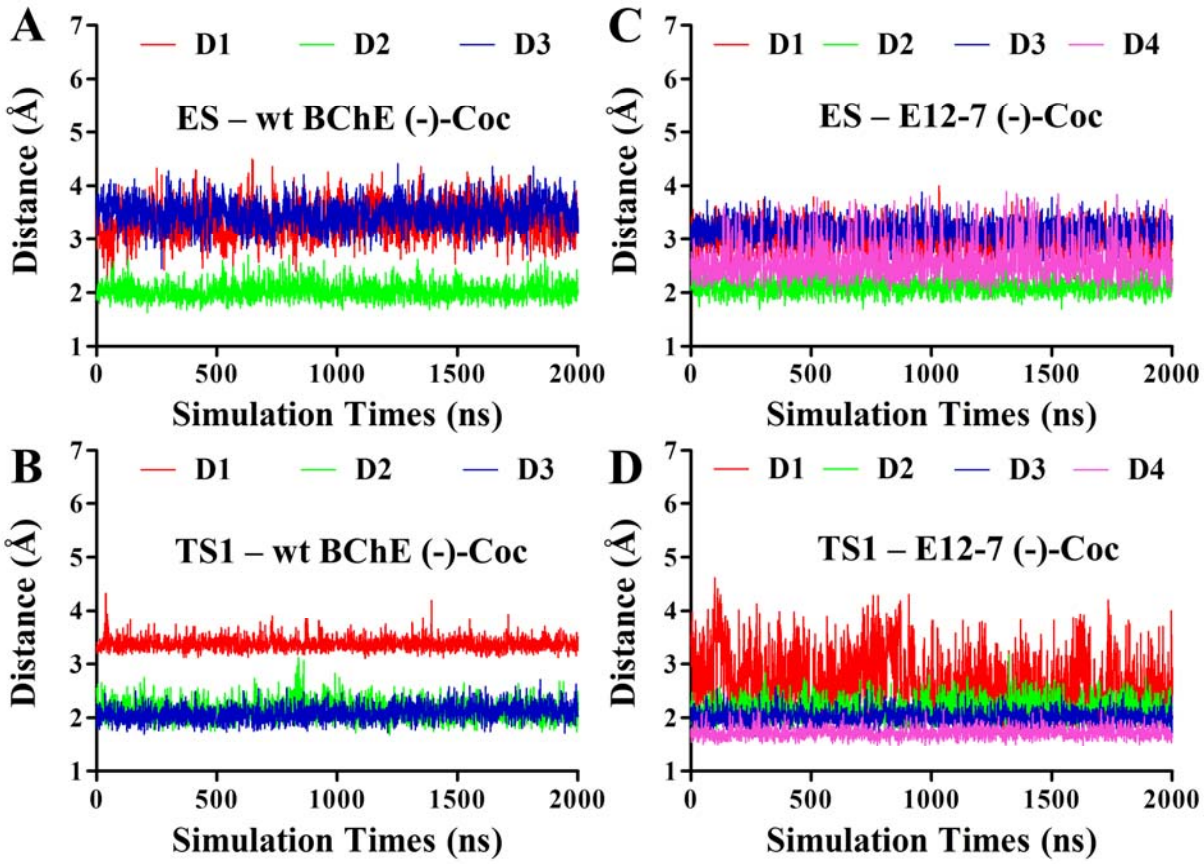

Figure S1. Key internuclear distances (in Å) versus the time in the simulated ES and TS1 structures for (-)-cocaine hydrolysis catalyzed by wt BChE (wild type BChE) or E12-7 (A199S/F227A/S287G/A328W/Y332G BChE). Traces D1, D2, and D3 refer to the distances between the carbonyl oxygen of (-)-cocaine and the NH hydrogen of G116, G117, and A199 (in wt BChE)/S199 (in E12-7), respectively. Trace D4 is the distance between the carbonyl oxygen of (-)-cocaine and the hydroxyl hydrogen of the S199 side chain in E12-7.

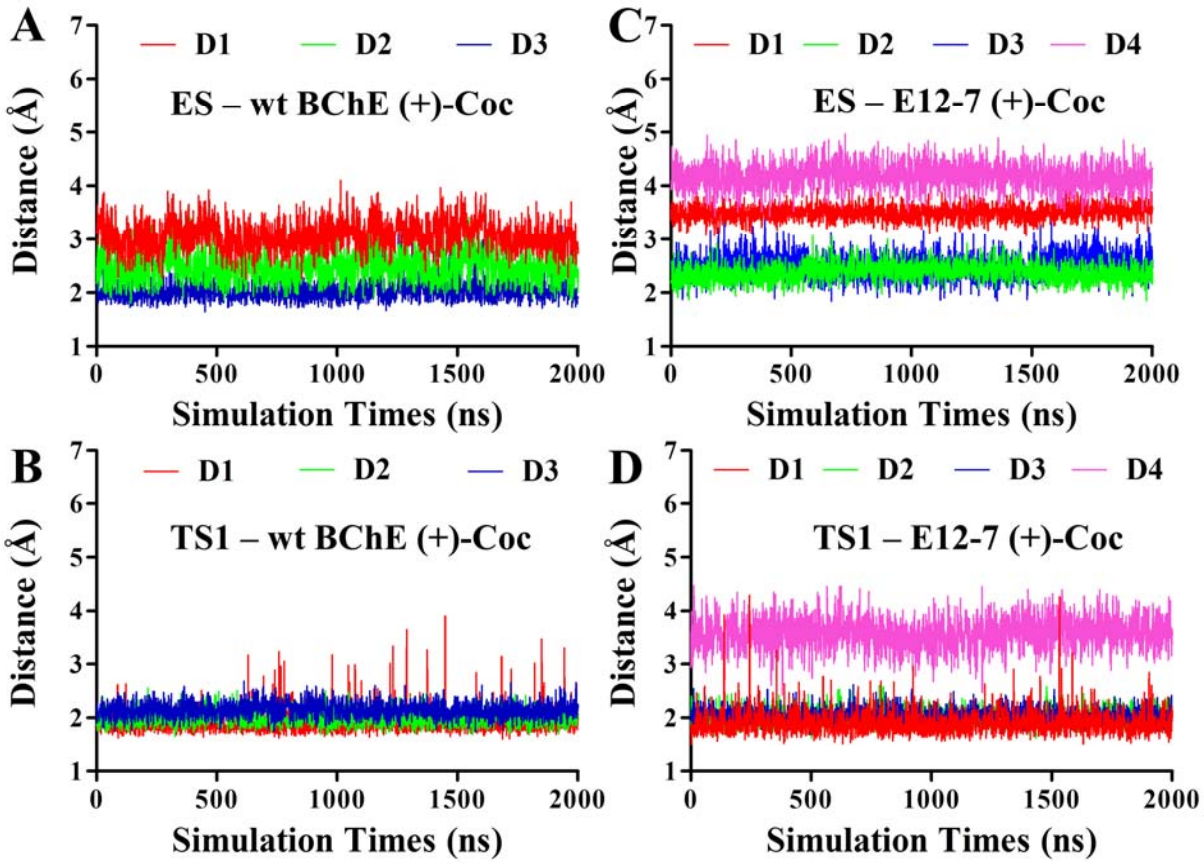

Figure S2. Key internuclear distances (in Å) versus the time in the simulated ES and TS1 structures for (+)-cocaine hydrolysis catalyzed by wt BChE (wild type BChE) or E12-7 (A199S/F227A/S287G/A328W/Y332G BChE). Traces D1, D2, and D3 refer to the distances between the carbonyl oxygen of (+)-cocaine and the NH hydrogen of G116, G117, and A199 (in wt BChE)/S199 (in E12-7), respectively. Trace D4 is the distance between the carbonyl oxygen of (+)-cocaine and the hydroxyl hydrogen of the S199 side chain in E12-7.

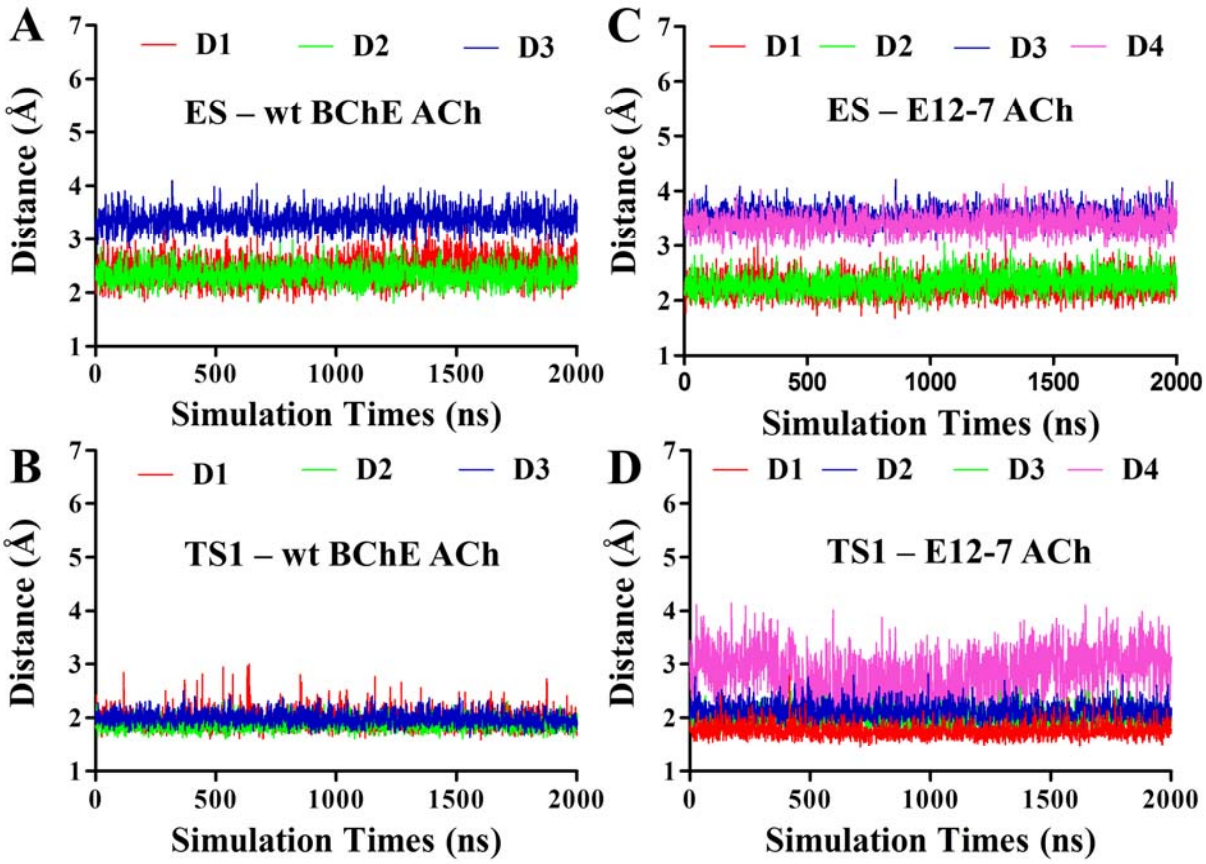

Figure S3. Key internuclear distances (in Å) versus the time in the simulated ES and TS1 structures for ACh (acetylcholine) hydrolysis catalyzed by wt BChE (wild type BChE) or E12-7 (A199S/F227A/S287G/A328W/Y332G BChE). Traces D1, D2, and D3 refer to the distances between the carbonyl oxygen of ACh and the NH hydrogen of G116, G117, and A199 (in wt BChE)/S199 (in E12-7), respectively. Trace D4 is the distance between the carbonyl oxygen of ACh and the hydroxyl hydrogen of the S199 side chain in E12-7.

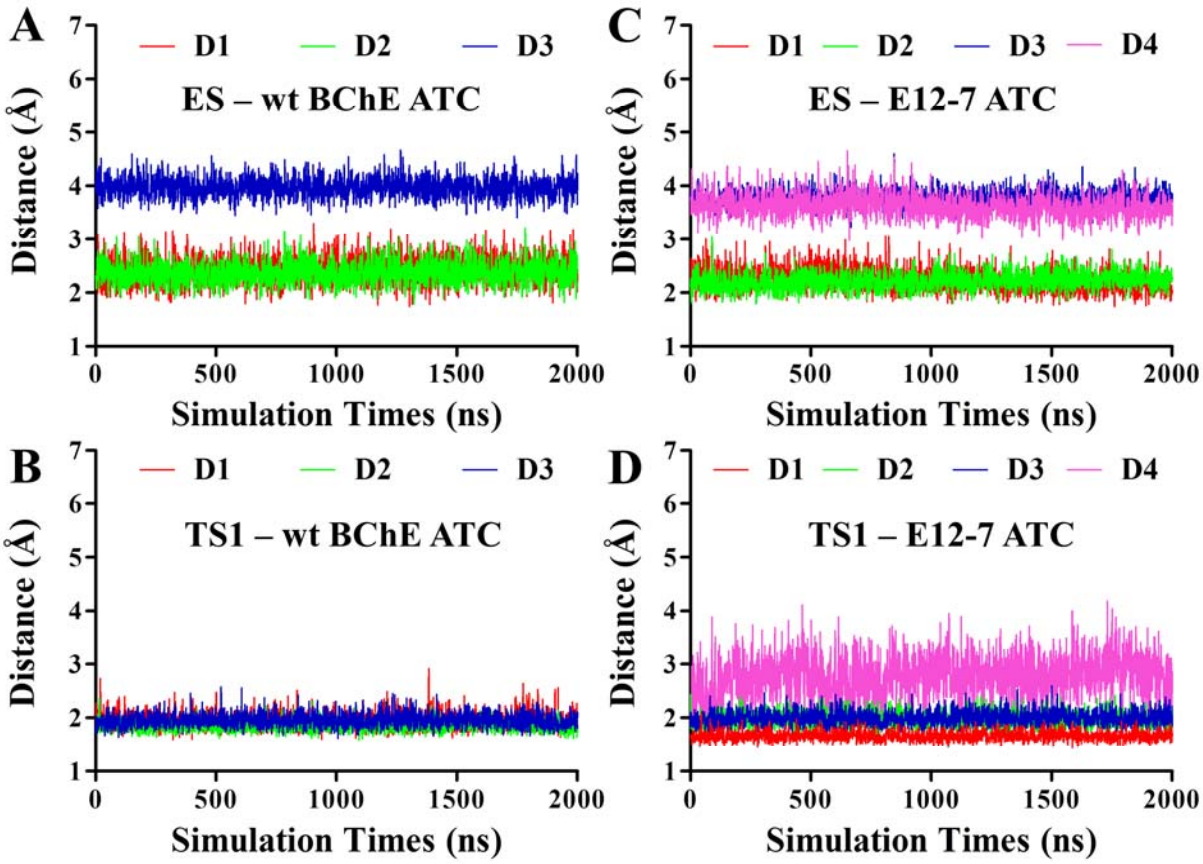

Figure S4. Key internuclear distances (in Å) versus the time in the simulated ES and TS1 structures for ATC (acetylthiocholine) hydrolysis catalyzed by wt BChE (wild type BChE) or E12-7 (A199S/F227A/S287G/A328W/Y332G BChE). Traces D1, D2, and D3 refer to the distances between the carbonyl oxygen of ATC and the NH hydrogen of G116, G117, and A199 (in wt BChE)/S199 (in E12-7), respectively. Trace D4 is the distance between the carbonyl oxygen of ATC and the hydroxyl hydrogen of the S199 side chain in E12-7.

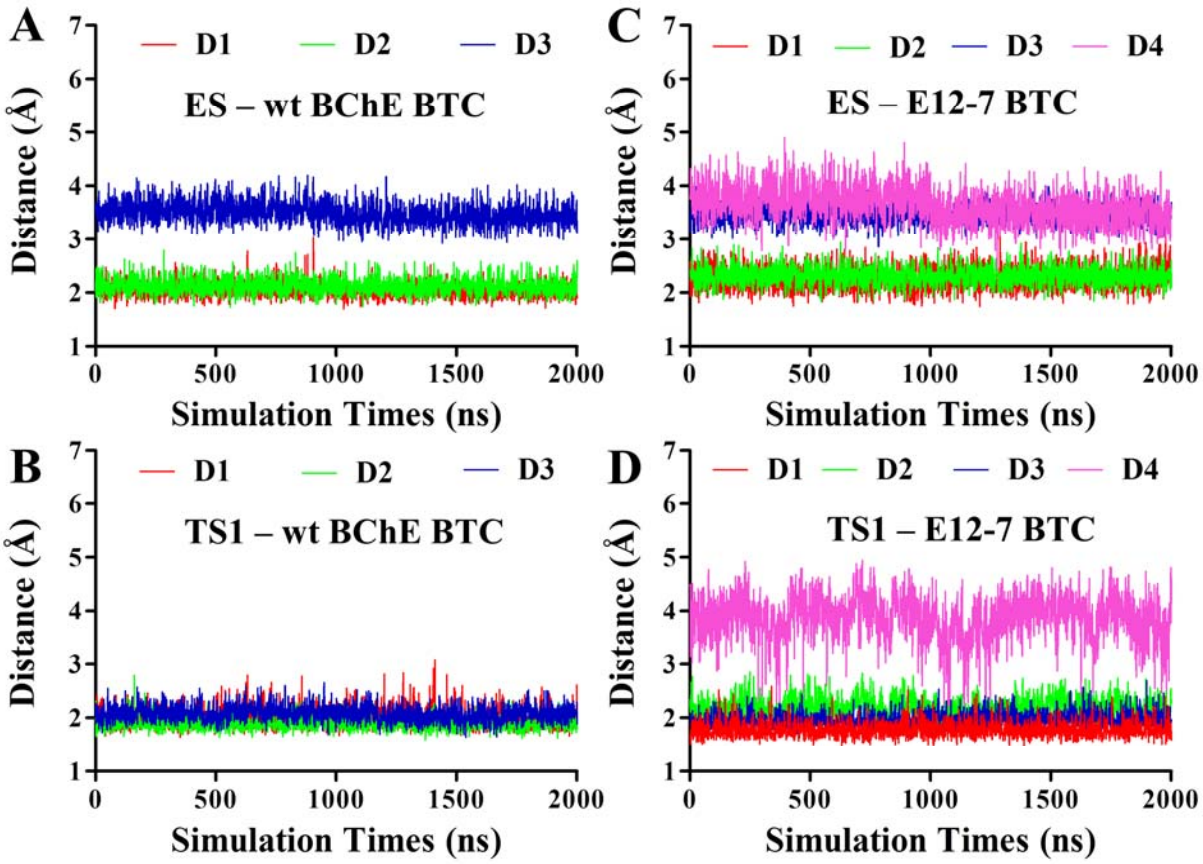

Figure S5. Key internuclear distances (in Å) versus the time in the simulated ES and TS1 structures for BTC (butyrylthiocholine) hydrolysis catalyzed by wt BChE (wild type BChE) or E12-7 (A199S/F227A/S287G/A328W/Y332G BChE). Traces D1, D2, and D3 refer to the distances between the carbonyl oxygen of BTC and the NH hydrogen of G116, G117, and A199 (in wt BChE)/S199 (in E12-7), respectively. Trace D4 is the distance between the carbonyl oxygen of BTC and the hydroxyl hydrogen of the S199 side chain in E12-7.