Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Linking inhibitor motions to proteolytic stability of sunflower trypsin inhibitor-1

Wanqing Wei, Jing Ma, Daiqian Xie and Yanzi Zhou[*]

Institute of Theoretical and Computational Chemistry, Laboratory of Mesoscopic Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

* To whom correspondence should be addressed. E-mail: zhouyz@nju.edu.cn.

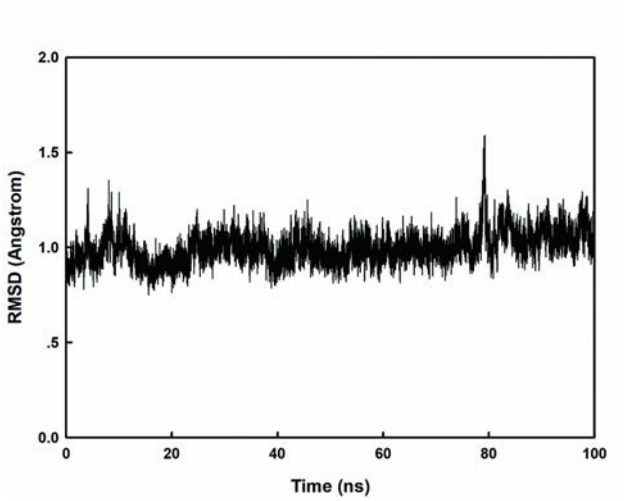
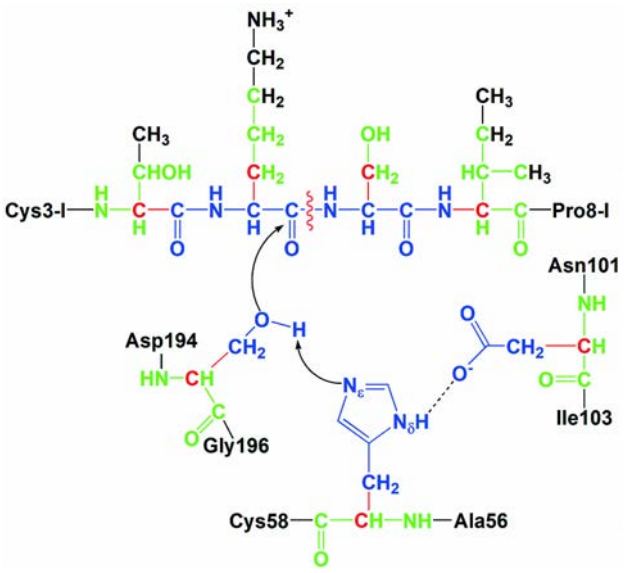
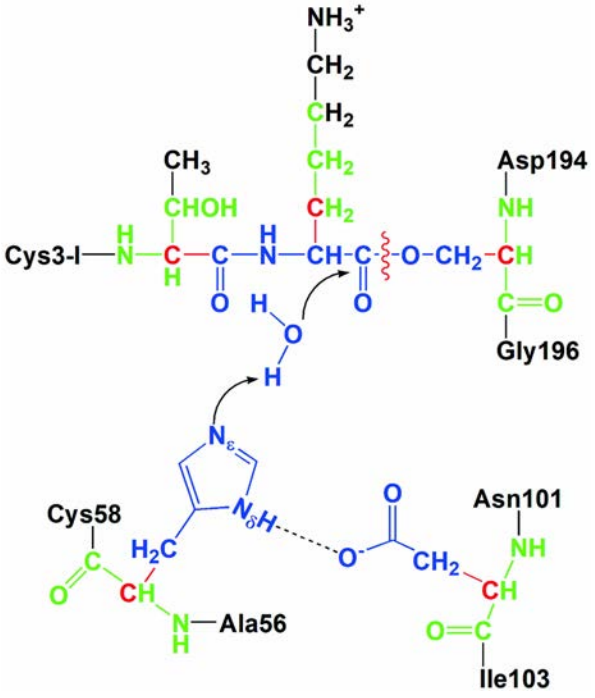


Fig. S1 The calculated RMSD of the backbone atoms of trypsin–SFTI-1 complex compared to the high resolution crystal structure from a 100 ns classic MD simulation.



(a)



(b)

Fig. S2 Illustration of the division of QM/MM system for (a) acylation reaction (b) deacylation reaction. All atoms colored in blue are QM atoms and assigned with 6-31+G* basis set, and the pseudo-atoms (colored in red) are treated with pseudo-bond parameters. All the left atoms are MM atoms that are treated with Amber99SB force field. The atoms connected with pseudo-atoms (colored in green) are treated as zero-charge atoms, and have no electrostatic interaction with QM atoms.

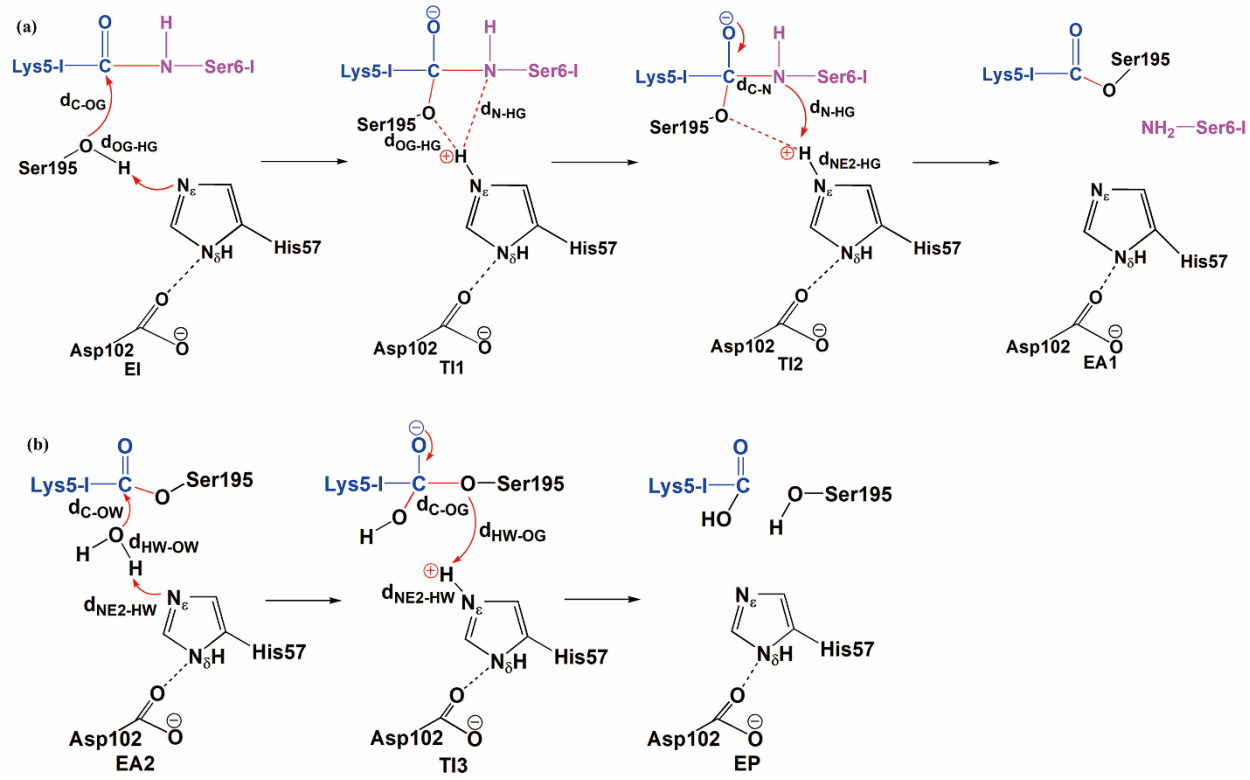


Fig. S3 Illustration of reaction mechanism for hydrolysis of SFTI-1 by trypsin and reaction coordinates chosen at each step of (a) acylation, (b) deacylation. "–I" refers to residues belonging to inhibitor SFTI-1. For the initial step of acylation reaction, the reaction coordinate is chosen as $d_{\text{OG-HG}} - d_{\text{C-OG}}$, which is the distance between O-H bond of Ser195 and the distance between attacking O of Ser195 and the carbon atom of scissile bond. The reaction coordinate of the second step is $d_{\text{OG-HG}} - d_{\text{N-HG}}$, the distances of two H-bonds formed with the proton. The reaction coordinate of the third step is $d_{\text{C-N}} - d_{\text{N-HG}} + d_{\text{NE2-HG}}$, in which $d_{\text{C-N}}$ means the distance between C and N of the scissile bond, $d_{\text{N-HG}}$ is the distance between N atom of scissile and H, and $d_{\text{NE2-HG}}$ is distance between H and the N atom of His57. For deacylation reaction, the reaction coordinates are $d_{\text{OW-HW}} - d_{\text{C-OW}}$ and $d_{\text{C-OG}} - d_{\text{HW-OG}} + d_{\text{NE2-HW}}$ for the two steps respectively.

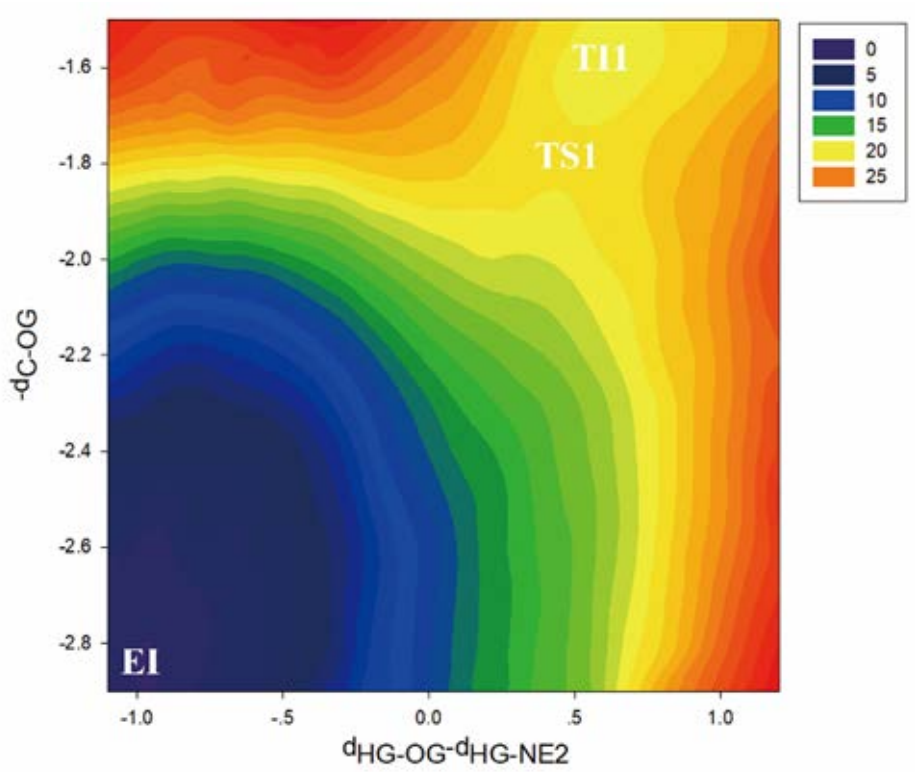


Fig. S4 The 2-dimensional free energy surface for the first step of acylation reaction for SFTI-1.

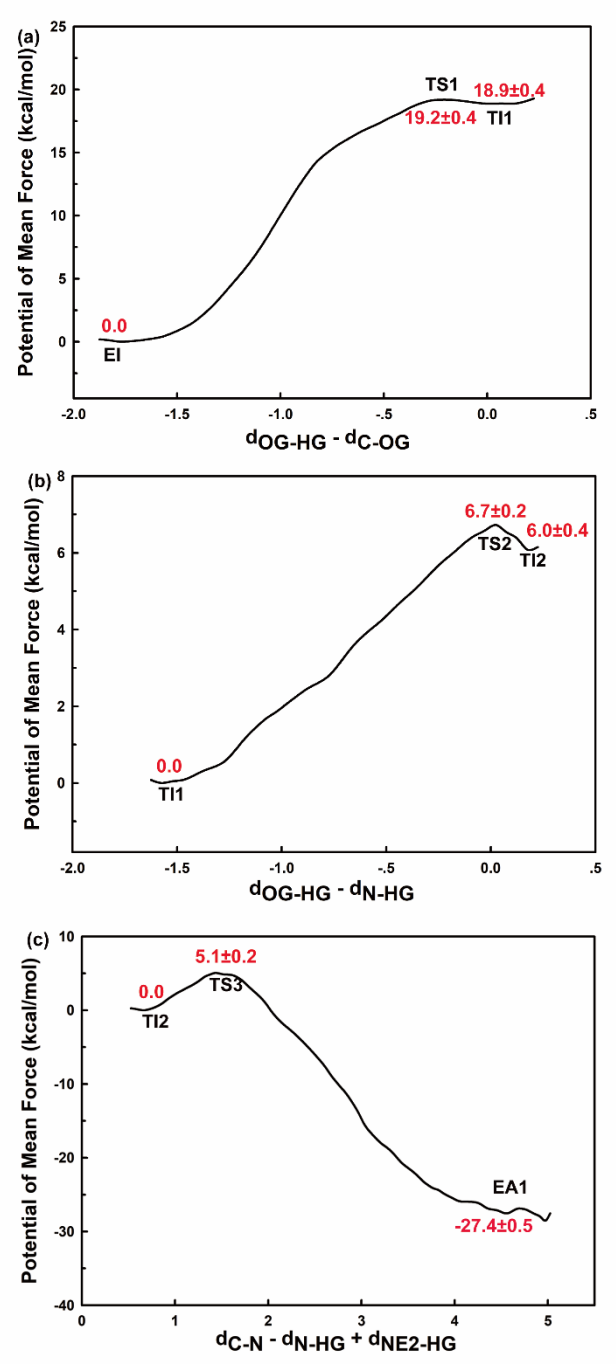


Fig. S5 The one-dimensional free energy curves for (a) the first step; (b) the second step; (c) the third step of acylation reaction for SFTI-1.

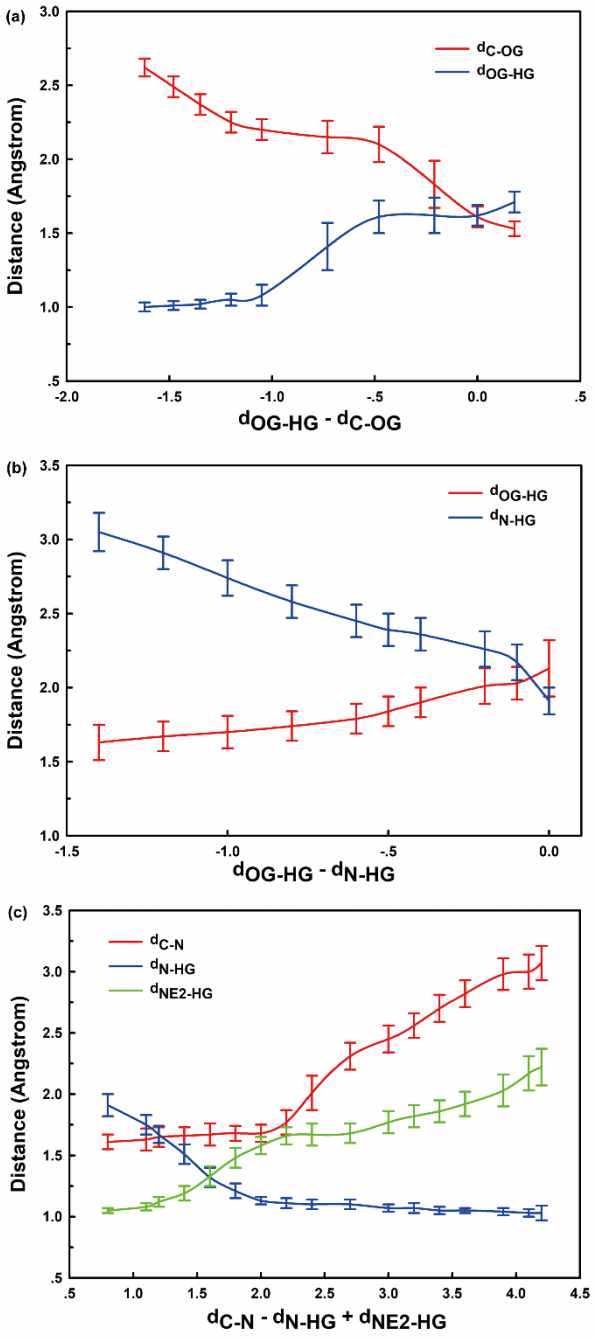


Fig. S6 The change of reaction coordinates for (a) the first step; (b) the second step; (c) the third step of acylation reaction for SFTI-1. Distance names are the same as defined in Figure S3.

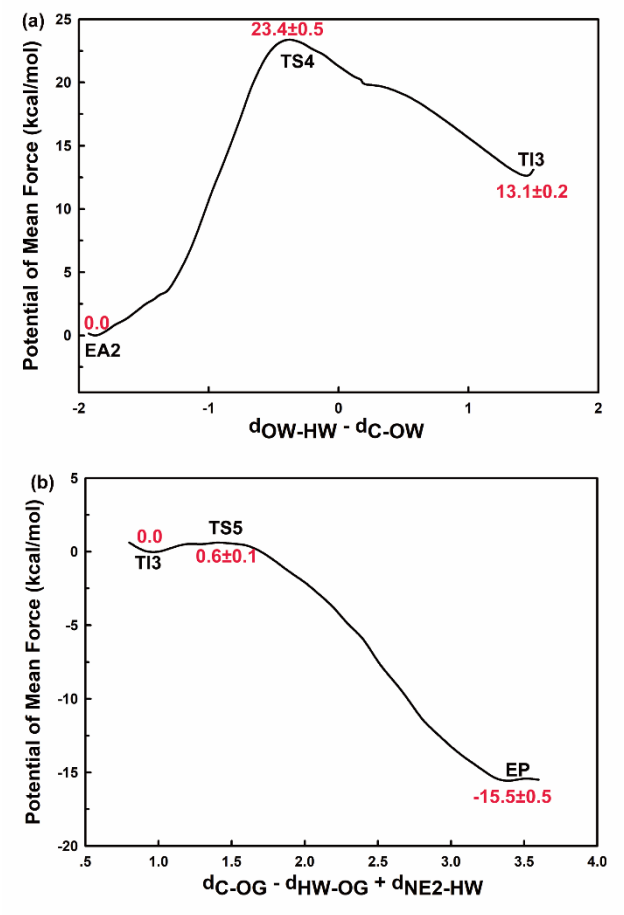


Fig. S7 The one-dimensional free energy curves for (a) the first step; (b) the second step of deacylation reaction for SFTI-1.

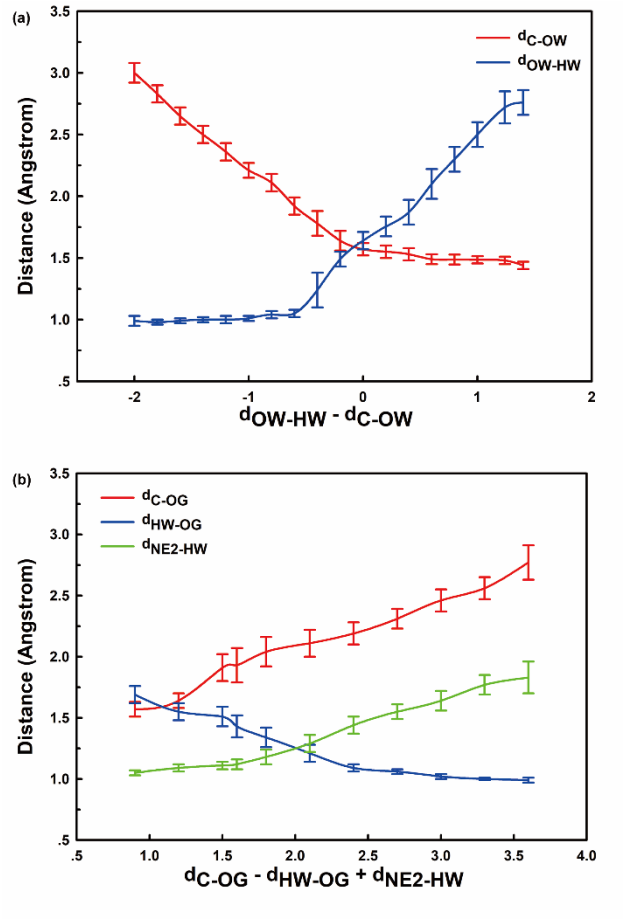


Fig. S8 The change of reaction coordinates for (a) the first step; (b) the second step of deacylation reaction for SFTI-1. Distance names are the same as defined in Figure S3.

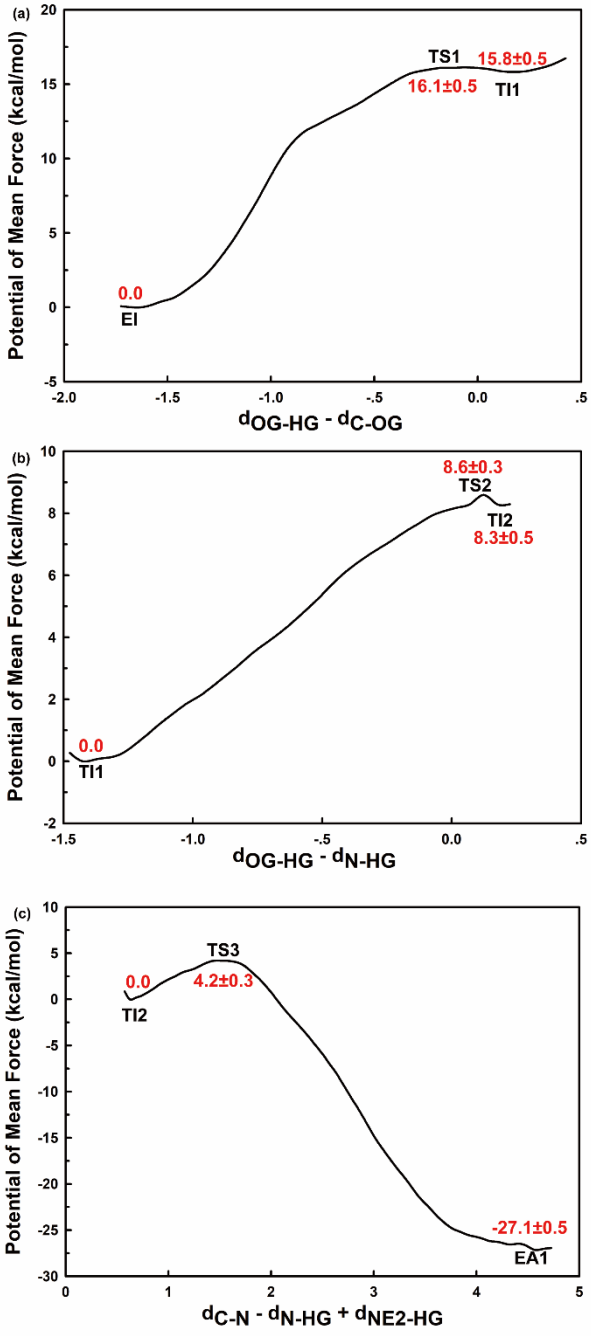


Fig. S9 The one-dimensional free energy curves for (a) the first step; (b) the second step; (c) the third step of acylation reaction for BiKF.

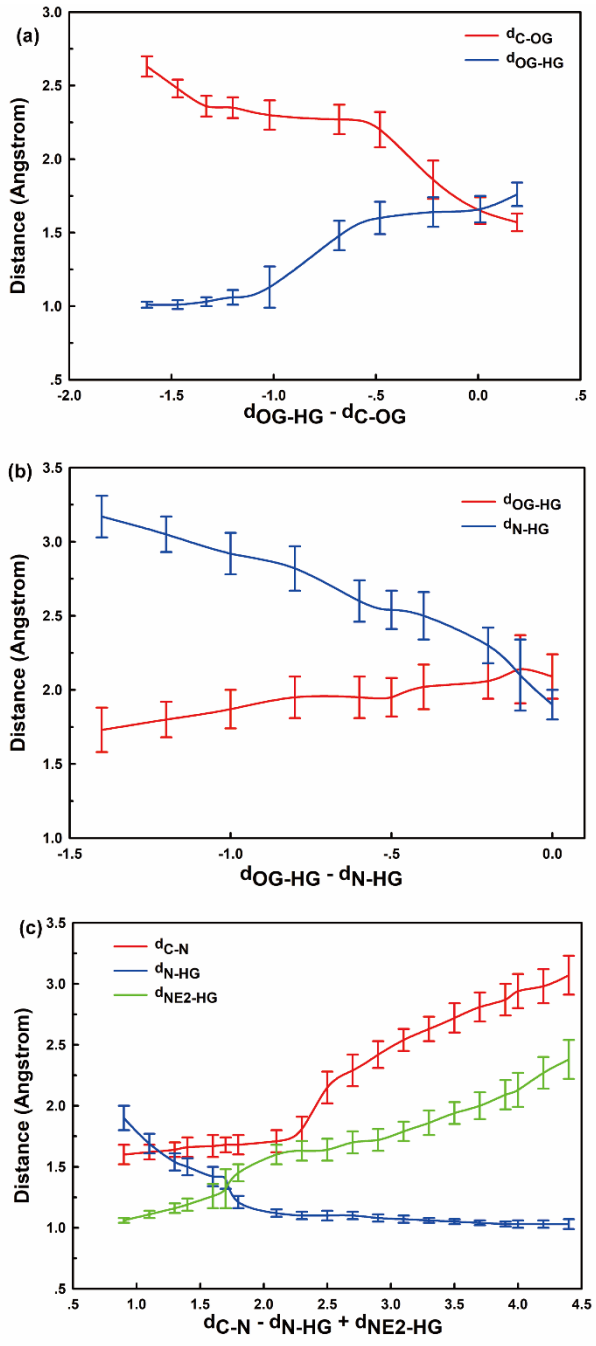


Fig. S10 The change of reaction coordinates for (a) the first step; (b) the second step; (c) the third step of acylation reaction for BiKF. Distance names are the same as defined in Figure S3.

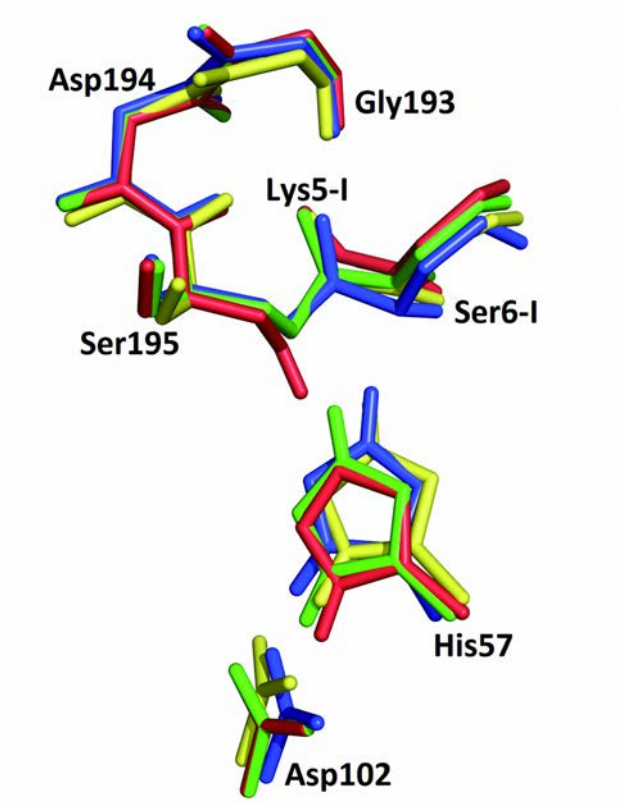


Fig. S11 Superposition of structures of the active region for tryspin-SFTI-1 at EI (in red), TI1 (in green), TI2 (in blue) and TS3 (in yellow) states in stick mode. "–I" means this residue belongs to inhibitor SFTI-1.

Table S1. List of key geometric parameters for the reactant, transition states, intermediate and acyl-enzyme of acylation reaction for trypsin-SFTI-1 based on QM/MM MD simulations.

Distance(Å)	EI	TS1	TI1	TS2	TI2	TS3	EA1
OG(S195)-C(K5-I)	2.62±0.07	1.83±0.16	1.55±0.07	1.50±0.05	1.49±0.05	1.47±0.05	1.33±0.03
HG(S195)-OG(S195)	1.00±0.02	1.62±0.12	1.63±0.12	2.03±0.11	2.13±0.19	2.29±0.12	2.71±0.18
HG(S195)-NE2(H57)	1.82±0.13	1.08±0.04	1.06±0.03	1.03±0.03	1.05±0.02	1.32±0.07	2.19±0.15
HG(S195)-N'(S6-I)	3.62±0.17	3.45±0.33	3.05±0.13	2.17±0.12	1.91±0.09	1.33±0.07	1.03±0.06
C(K5-I)-N'(S6-I)	1.37±0.02	1.45±0.05	1.53±0.05	1.57±0.06	1.61±0.06	1.68±0.09	3.07±0.14
C(K5-I)-O(K5-I)	1.23±0.02	1.26±0.02	1.29±0.03	1.28±0.02	1.29±0.03	1.27±0.03	1.22±0.02
ND1(H57)-OD1(D102)	2.92±0.13	3.06±0.29	2.86±0.15	3.20±0.29	3.48±0.18	3.54±0.24	3.14±0.26
ND1(H57)-OD2(D102)	3.16±0.22	3.03±0.18	3.12±0.17	2.79±0.15	2.77±0.14	3.03±0.19	3.20±0.28
HD1(H57)-OD1(D102)	1.91±0.15	2.08±0.35	1.82±0.17	2.29±0.37	2.63±0.19	2.69±0.28	2.19±0.31
HD1(H57)-OD2(D102)	2.48±0.23	2.25±0.27	2.43±0.18	1.90±0.30	1.76±0.16	2.06±0.23	2.39±0.32
HD1(H57)-ND1(H57)	1.04±0.03	1.04±0.03	1.05±0.03	1.06±0.03	1.06±0.04	1.04±0.03	1.03±0.03
N(S195)-O(K5-I)	2.99±0.13	2.88±0.12	3.00±0.13	2.99±0.13	2.94±0.11	2.87±0.10	2.98±0.12
N(D194)-O(K5-I)	3.26±0.18	3.25±0.20	3.49±0.17	3.52±0.18	3.47±0.17	3.60±0.19	3.41±0.19
N(G193)-O(K5-I)	2.87±0.11	2.78±0.08	2.78±0.08	2.77±0.08	2.77±0.07	3.06±0.13	2.87±0.11
∠NE2(H57)-HG(S195)-N'(S6-I)/degree	123.6±6.5	135.0±6.0	120.7±5.3	155.1±7.6	160.1±9.3	172.0±4.3	165.2±8.0
Torsion ω/degree	190.6±6.2	179.6±6.1	163.4±5.0	162.6±5.6	146.9±5.3	143.8±6.0	148.0±8.6

Table S2. List of key geometric parameters for the reactant, transition states, intermediate and acyl-enzyme of acylation reaction for trypsin-BiKF based on QM/MM MD simulations.

Distance(Å)	EI	TS1	TI1	TS2	TI2	TS3	EA1
OG(S195)-C(K5-I)	2.62±0.08	1.85±0.14	1.56±0.06	1.51±0.05	1.47±0.04	1.45±0.05	1.33±0.03
HG(S195)-OG(S195)	1.01±0.02	1.65±0.10	1.80±0.12	2.14±0.23	2.09±0.16	2.33±0.15	2.70±0.19
HG(S195)-NE2(H57)	1.77±0.13	1.07±0.03	1.05±0.03	1.04±0.03	1.06±0.02	1.29±0.09	2.38±0.16
HG(S195)-N'(S6-I)	3.53±0.19	3.40±0.24	3.05±0.12	2.29±0.24	1.90±0.10	1.38±0.12	1.03±0.04
C(K5-I)-N'(S6-I)	1.36±0.02	1.44±0.04	1.49±0.03	1.55±0.06	1.66±0.08	1.72±0.15	3.07±0.16
C(K5-I)-O(K5-I)	1.23±0.02	1.26±0.02	1.29±0.02	1.29±0.03	1.28±0.03	1.27±0.03	1.22±0.02
ND1(H57)-OD1(D102)	3.00±0.20	2.94±0.24	2.99±0.26	3.42±0.20	3.48±0.21	3.53±0.22	3.43±0.28
ND1(H57)-OD2(D102)	3.20±0.27	3.09±0.20	3.08±0.19	2.78±0.12	2.81±0.14	2.86±0.13	3.09±0.26
HD1(H57)-OD1(D102)	1.99±0.23	1.93±0.28	2.00±0.31	2.57±0.22	2.62±0.23	2.69±0.24	2.53±0.34
HD1(H57)-OD2(D102)	2.48±0.29	2.35±0.27	2.28±0.26	1.77±0.17	1.83±0.19	1.86±0.17	2.16±0.32
HD1(H57)-ND1(H57)	1.04±0.03	1.05±0.04	1.05±0.03	1.06±0.03	1.05±0.04	1.04±0.03	1.03±0.02
N(S195)-O(K5-I)	3.01±0.15	2.80±0.09	2.87±0.10	2.92±0.12	3.00±0.11	3.01±0.14	2.93±0.11
N(D194)-O(K5-I)	3.22±0.17	3.20±0.17	3.37±0.15	3.43±0.18	3.57±0.20	3.60±0.19	3.39±0.18
N(G193)-O(K5-I)	2.82±0.08	2.77±0.07	2.77±0.08	2.78±0.09	2.85±0.10	2.82±0.10	2.92±0.15
∠NE2(H57)-HG(S195-N'(S6-I)/degree	127.2±6.1	136.0±6.0	143.1±5.0	158.9±7.8	159.7±8.8	170.4±6.2	166.4±7.4
Torsion ω/degree	187.8±6.2	178.4±6.7	175.4±5.2	159.2±6.7	147.8±5.1	140.5±5.5	149.7±10.8

Table S3. Intermolecular hydrogen bonds at EI state for SFTI-1 and its analogs in trypsin from 100 ns classic MD simulations. A hydrogen bond is counted if the donor-acceptor distance is less than 3.0 Å and the angle between acceptor-hydrogen-donor is less than 135^{o}. The values indicate percentage of total simulation time for which a given H-bond was present. An interaction is recorded only if it was present for >10% in at least one simulation. ("–I" means this residue belongs to the inhibitors.)

Acceptor	Donor	Inhibitor				
		SFTI-1	BiKF	Analog-1	Analog-2	Analog-3
Gly_1-I@O	Ser_217@OG	0.18	-	0.51	0.30	0.39
Pro_1-I@O	Ser_217@OG	-	0.15	-	-	-
Gly_1-I@O	Gly_219@N	-	-	-	-	0.11
Asn_97@O	Arg_2-I@NH1/NH2	0.17	-	0.43	0.41	-
Gln_175@OE1	Arg_2-I@NH1	-	-	-	-	0.21
Cys_3-I@O	Gly_216@N	0.37	0.52	0.39	-	-
Abu_3-I@O	Gly_216@N	-	-	-	0.42	0.34
Gly_216@O	Cys_3-I@N	0.56	0.57	0.49	-	-
Gly_216@O	Abu_3-I@N	-	-	-	0.63	0.47
Thr_4-I@O	Gln_192@NE2	0.73	0.11	0.38	-	-
Ser_190@O	Lys_5-I@NZ	0.64	0.82	0.67	0.49	0.58
Lys_5-I@O	Gly_193@N	0.88	0.82	0.80	0.86	0.81
Lys_5-I@O	Ser_195@N	0.49	0.38	0.52	0.52	0.44
Ser_214@O	Lys_5-I@N	0.36	-	0.29	0.12	0.32
Gly_219@O	Lys_5-I@NZ	-	0.11	-	-	-
Ile_7-I@O	Tyr_39@OH				0.28	
Phe_41@O	Ile_7-I@N	0.11	-	-	0.14	-
Ser_96@O	Asp_14-I@N	-	-	-	-	0.37
Asp_14-I@O/OD1/OD2	Asn_97@ND2	-	-	-	-	0.75
Asp_14-I@OD1/OD2	Gln_175@NE2	0.50	-	0.25	0.47	-
Pro_16-I@O	Gln_175@NE2	-	0.39	-	-	-
Total		5.00	3.86	4.73	4.64	4.79

Table S4. Distances between heavy atoms of intramolecular hydrogen bonds for SFTI-1 and BiKF at EI state from QM/MM MD simulations.

Distance(Å)	SFTI-1	BiKF
N(T4-I) - O(I10-I)	2.96±0.13	3.10±0.16
OG(T4-I) - N(I10-I)	2.90±0.10	3.03±0.16
OG(T4-I) - OG(S6-I)	2.76±0.12	2.78±0.12
OG(S6-I) - O(P8-I)	3.57±0.30	3.72±0.27
N(G1-I) - O(F12-I)[a]	3.09±0.21	-
O(R2-I) - N(F12-I)[a]	2.93±0.11	-
NE(R2-I) - OD1(D14-I)[a]	2.77±0.09	-
NH2(R2-I) - OD2(D14-I)[a]	2.77±0.10	-
O(I2-I)-N(T12-I)[b]	-	2.95±0.11
N(I2-I)-OG(T12-I)[b]	-	3.02±0.15
OG(T12-I)-OG(S14-I)[b]	-	2.87±0.15

[a] These hydrogen bonds are within SFTI-1;

[b] These hydrogen bonds are within BiKF.

Table S5. Intramolecular hydrogen bonds at EI state for the analogs and the wild type of SFTI-1 from 100 ns classic MD simulations. The values indicate percentage of total simulation time for which a given H-bond was present. An interaction is recorded only if it was present for >10% in at least one simulation.

Acceptor	Donor	Occupancy			
		SFTI-1	analog-1	analog-2	analog-3
Arg_2-I@O	Arg_2-I@NH1	-	-	-	0.11
Arg_2-I@O	Phe_12-I@N	0.70	0.74	0.59	-
Thr_4-I@OG1	Ile_10-I@N	0.51	0.61	0.54	0.29
Ser_6-I@OG	Thr_4-I@OG1	0.50	0.46	0.45	0.63
Pro_8-I@O	Ser_6-I@OG	0.12	0.21	0.15	0.22
Ile_10-I@O	Thr_4-I@N	0.66	0.73	0.45	0.71
Ile_10-I@O	Phe_12-I@N	-	-	-	0.85
Phe_12-I@O	Gly_1-I@N	0.30	-	0.31	-
Phe_12-I@O	Arg_2-I@N	-	0.20	-	-
Asp_14-I@O	Arg_2-I@N	-	0.15	-	-
Asp_14-I@OD1	Arg_2-I@NE/NH2	0.78	0.35	0.42	-
Asp_14-I@OD2	Arg_2-I@NE/NH2	0.91	0.33	0.36	-
Total		4.48	3.78	3.27	2.81