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Supporting Information for

Molecular insight into chymotrypsin inhibitor 2 resisting proteolytic degradation

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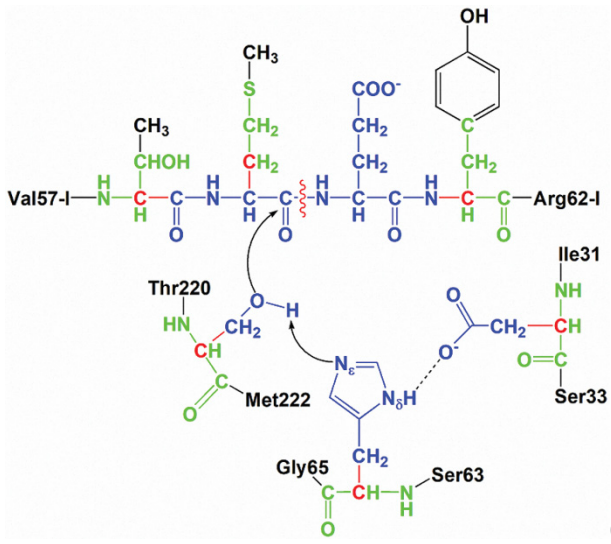

Fig. S1 Illustration of the division of QM/MM system for (a) acylation reaction (b) deacylation reaction. All atoms colored in blue are QM atoms and assigned with 6-31+G* basis set, and the pseudo-atoms (colored in red) are treated with pseudo-bond parameters. All the left atoms are MM atoms that are 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.

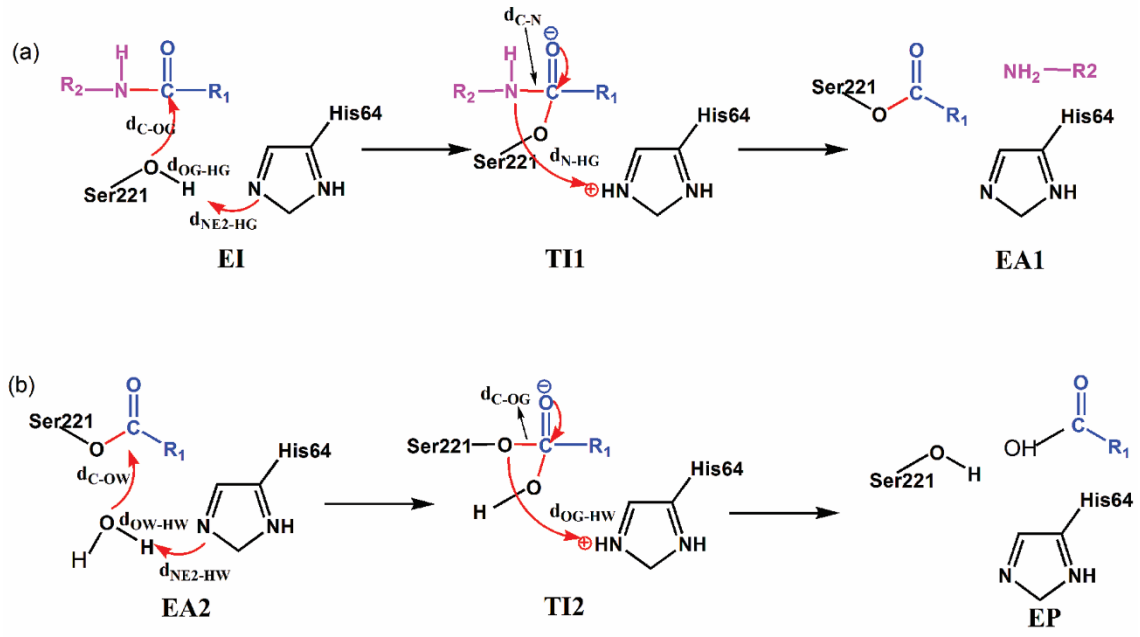

Fig. S2 Illustration of reaction mechanism for hydrolysis of CI2 by subtilisin and reaction coordinates chosen at each step of (a) acylation, (b) deacylation.

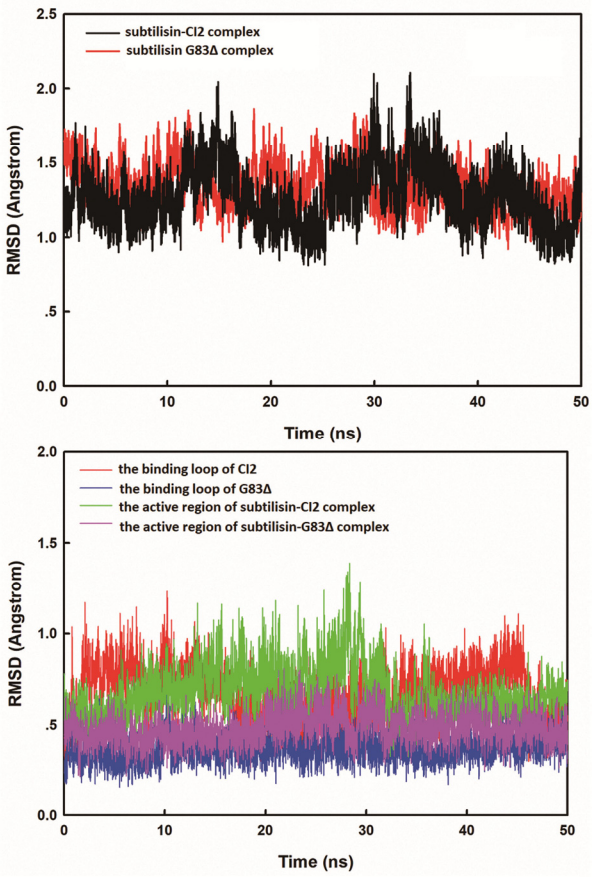

Fig. S3 The calculated RMSDs of the backbone heavy atoms of subtilisin-CI2 and subtilisin-G83Δ complexes compared to the high-resolution crystal structure. (a) RMSDs of the two complexes. (b) RMSDs of the binding loop and residues in the active region, which includes the scissile peptide bond (Met59, Glu60) and the catalytic triad (Ser221, His64 and Asp32).

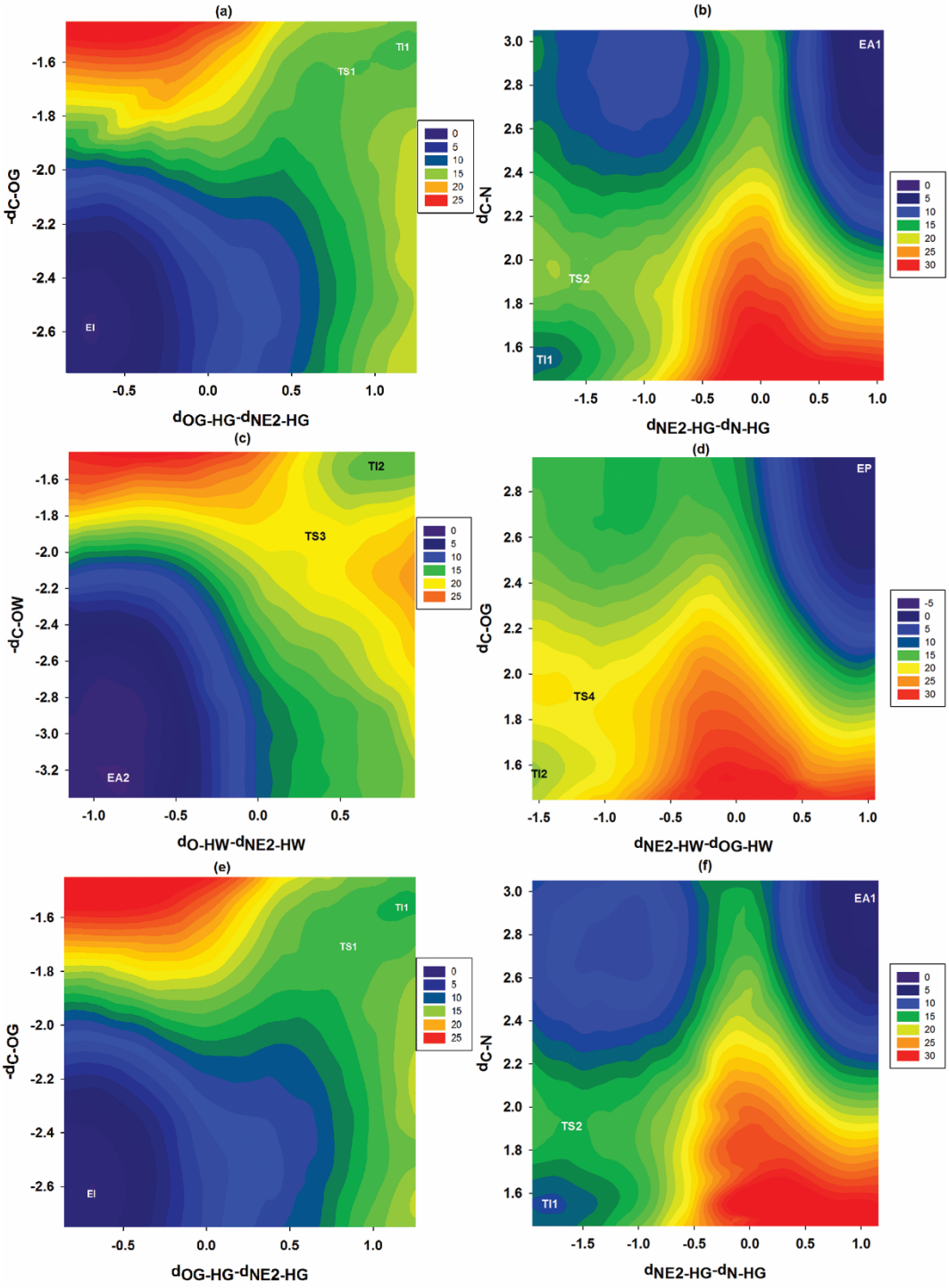

Fig. S4 The free energy surfaces for the whole proteolysis reaction of CI2: (a) the first step of acylation; (b) the second step of acylation; (c) the first step of deacylation; (d) the second step of deacylation; and for the acylation of G83Δ: (e) the first step; (f) the second step. Energies are in kcal/mol and distances are in Å.

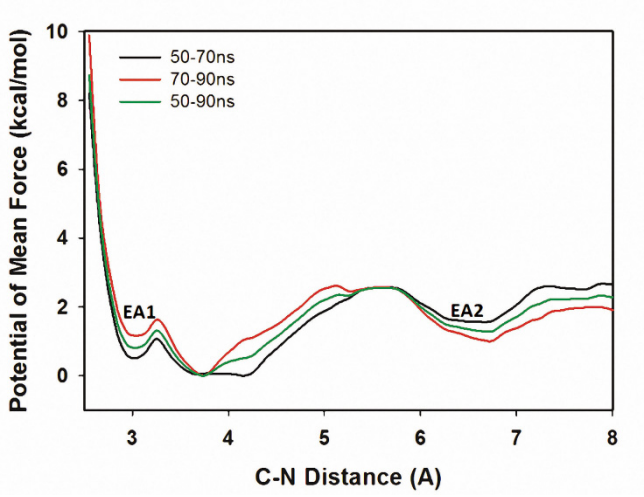

Fig. S5 The potential of mean force for leaving of C-terminal part of MCTI-A. The reaction coordinate is chosen as the length of scissile bond C-N.

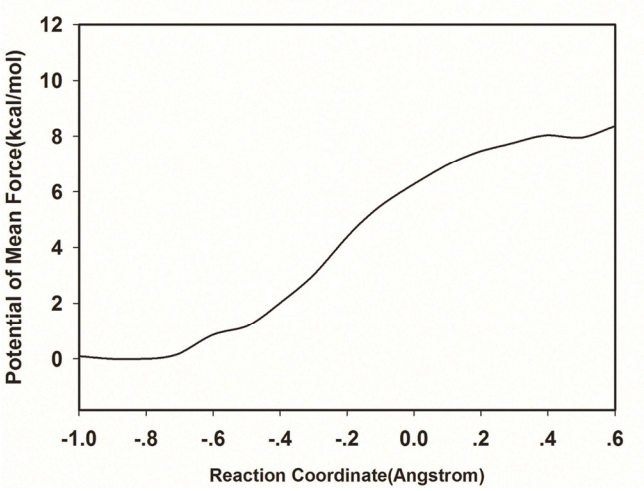

Fig. S6 Free energy profile for the proton transfer between His57 and Asp102 at the transition state (TS2*) of the acylation stage for the MCTI-A. The reaction coordinate is $d_{ND1\text{-}HD1} - d_{OD1\text{-}HD1}$. From left to right, the proton transfers from His57 to the carboxyl oxygen of Asp102.

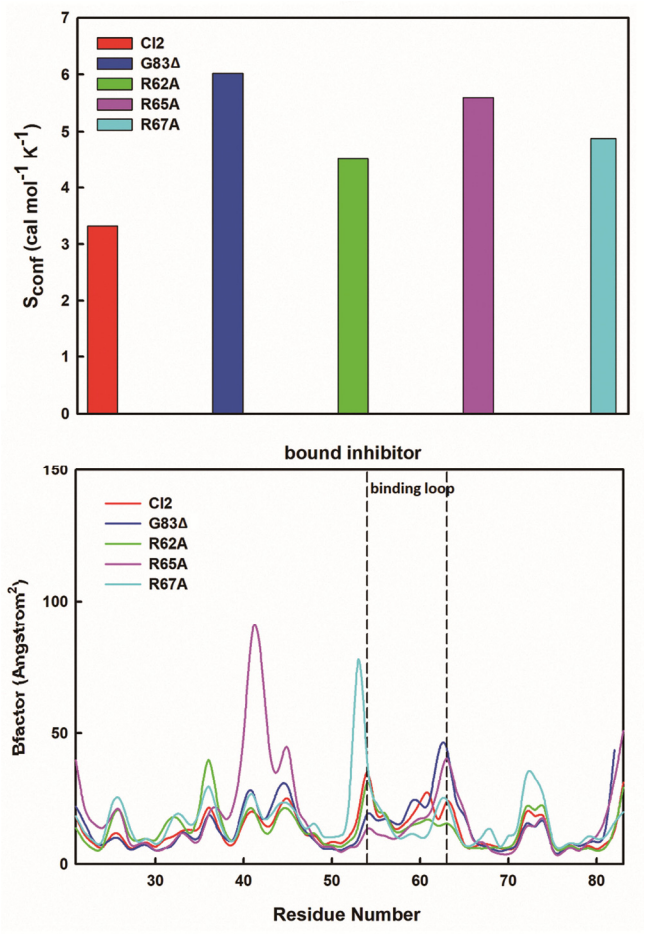

Fig. S7 (a) The total conformational entropies for CI2 and its mutants at EI state from 50 ns classic MD simulations; (b) Calculated B factors for backbone of CI2 and its mutants at EI state from 50 ns classic MD simulations.

Table S1. The intermolecular hydrogen bonds between subtilisin and the leaving group of CI2 and its mutants at EI state from 50 ns classical MD simulations. The values indicate fraction 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 refers to residues belonging to inhibitors.)

		Fraction				
Acceptor	Donor	CI2	G83Δ	R62A	R65A	R67A
ASN_62@O	ARG_62-I@NH2	--	0.64	--	--	--
ASN_62@OD1	ARG_65-I@NH1	--	0.13	--	--	--
SER_130@OG	GLN_78-I@NE2	--	--	--	0.15	--
GLU_156@OE2	ARG_81-I@NH2	0.51	0.27	0.80	--	0.33
GLU_156@OE1	ARG_81-I@NE/NH1	0.43	0.25	0.74	0.27	0.20
GLU_156@OE1	ARG_81-I@NH2	0.35	0.29	0.14	--	0.53
GLU_156@OE2	ARG_81-I@NE/NH1	0.24	0.26	0.15	0.16	0.49
ASN_218@O	TYR_61-I@N	0.88	0.72	0.85	0.70	0.86
GLU_60-I@OE2	ASN_62@ND2	--	--	--	--	--
GLU_60-I@OE1	TYR_217@OH	--	--	0.11	0.29	--
GLU_60-I@OE2	TYR_217@OH	--	--	--	0.25	--
TYR_61-I@O	ASN_218@ND2	--	0.37	--	--	--
GLN_78-I@OE1	SER_130@OG	--	--	--	0.17	--
Total		2.41	2.17	2.79	2.00	2.40

Table S2. The intramolecular hydrogen bonds between C-terminal and N-terminal parts for CI2 and its mutants at EI state from 50 ns classical MD simulations. The values indicate fraction 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.

		Fraction				
Acceptor	Donor	CI2	G83Δ	R62A	R65A	R67A
THR_22-I@O	VAL_82-I@N	0.55	0.37	0.53	0.43	0.53
GLU_23-I@OE1	ARG_81-I@NE	--	--	--	0.29	--
GLU_23-I@OE2	ARG_81-I@NE	--	--	--	0.53	0.13
GLU_23-I@OE1	ARG_81-I@NH2	--	--	--	0.20	--
GLU_23-I@OE2	ARG_81-I@NH2	--	--	--	0.43	--
GLY_29-I@O	ASN_75-I@ND2	0.45	--	--	--	--
LYS_30-I@O	ILE_76-I@N	0.79	0.75	0.68	0.81	0.65
GLN_47-I@O	VAL_66-I@N	0.61	0.62	0.84	0.73	0.74
ILE_49-I@O	LEU_68-I@N	0.89	0.78	0.88	0.57	0.83
LEU_51-I@O	VAL_70-I@N	0.61	0.58	0.66	0.48	--
THR_58-I@O	GLU_60-I@N	--	0.14	--	--	--
THR_58-I@O	ARG_67-I@NH1	0.72	0.79	0.20	0.15	--
THR_58-I@O	ARG_67-I@NH2	--	0.29	--	--	--
THR_58-I@OG1	ARG_67-I@NH2	0.53	0.42	0.11	--	--
GLU_60-I@OE1	THR_58-I@OG1	--	--	0.22	--	0.11
GLU_60-I@OE2	THR_58-I@OG1	0.18	--	0.45	0.24	0.11
ASP_64-I@O	GLN_47-I@N	0.79	0.38	0.55	0.28	0.63

ASP_64-I@OD1	GLN_47-I@NE2	--	--	--	0.21	--
VAL_66-I@O	ILE_49-I@N	0.22	0.38	0.45	0.62	0.44
LEU_68-I@O	LEU_51-I@N	0.78	0.70	0.63	0.19	0.81
VAL_70-I@O	VAL_53-I@N	0.51	0.16	0.43	0.69	--
ASP_74-I@OD1	SER_31-I@OG	0.11	--	--	--	--
ASP_74-I@OD2	SER_31-I@OG	0.14	--	--	--	--
ASP_74-I@O	VAL_32-I@N	0.34	--	--	--	0.19
ILE_76-I@O	GLY_29-I@N	0.78	0.74	0.78	0.74	0.39
PRO_80-I@O	TRP_24-I@N	0.46	0.52	0.49	0.26	0.45
Total1[a]		9.46	7.61	7.87	7.86	6.00
Total2[b]		11.87	9.78	10.66	9.86	8.40

[a] Total1 refers to sum of the fractions of intermolecular hydrogen bonds;

[b] Total2 refers to sum of the fractions of intramolecular and intermolecular hydrogen bonds.