Supporting Information

First QM/MM Studies of Inhibition Mechanism of Cruzain by Peptidyl Halomethyl Ketones.

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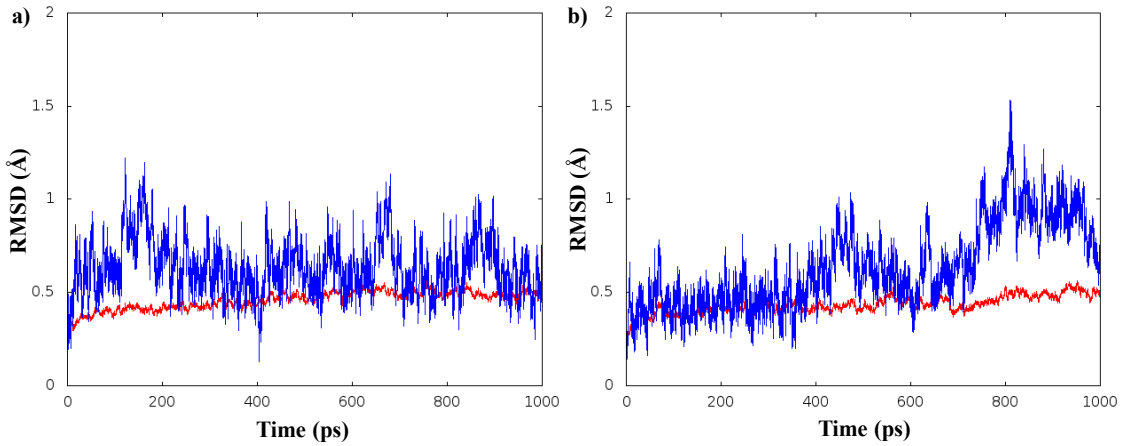

Figure S1. Time evolution of the root-mean-square-deviation (RMSD) along the QM/MM MD simulation for the backbone atoms of the protein (red line) and atoms of inhibitor (blue line) for (a) the Bz-Tyr-Ala-CH_2F and (b) the Bz-Tyr-Ala-CH_2Cl systems. Simulations performed on the protein-inhibitor bonded state (products).

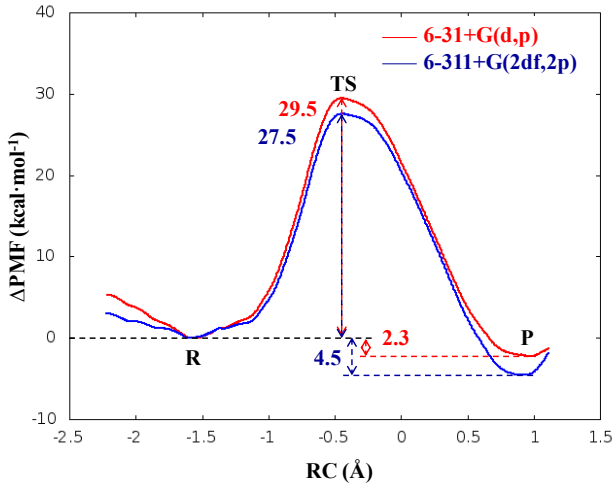

Figure S2. 1D-PMFs for cruzain inhibition by PFK through mechanism I computer at M062X/MM level with 6-31+G(d,p) and 6-311+G(2df,2p) basis set. RC corresponds to d(F-CM)-d(SG-CM).

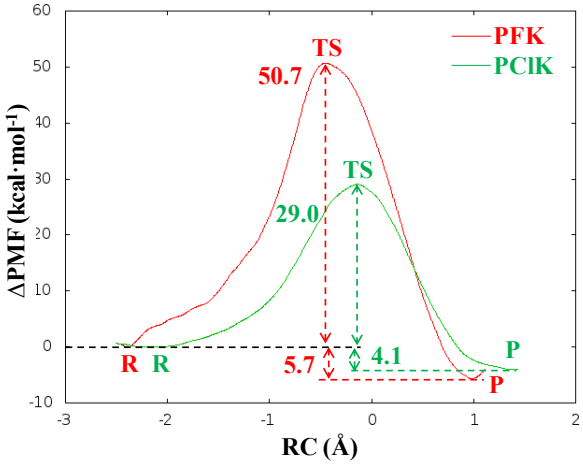

Figure S3. Free energy profiles of the cruzain inhibition with PHKs through mechanism I computed at AM1d/MM level. RC corresponds to d(X-CM)-d(SG-CM), with X: F, Cl.

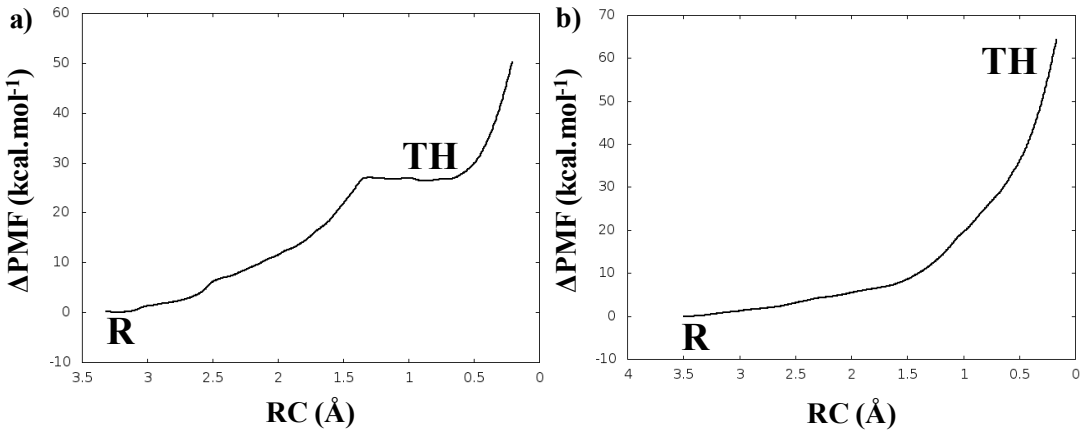

Figure S4. PMFs for the formation of TH intermediate from reactants computed at AM1d/MM level: a) Bz-Tyr-Ala-CH_2F inhibitor; and b) Bz-Tyr-Ala-CH_2Cl inhibitor. RC corresponds to d(SG-CT)-d(OT-CT).

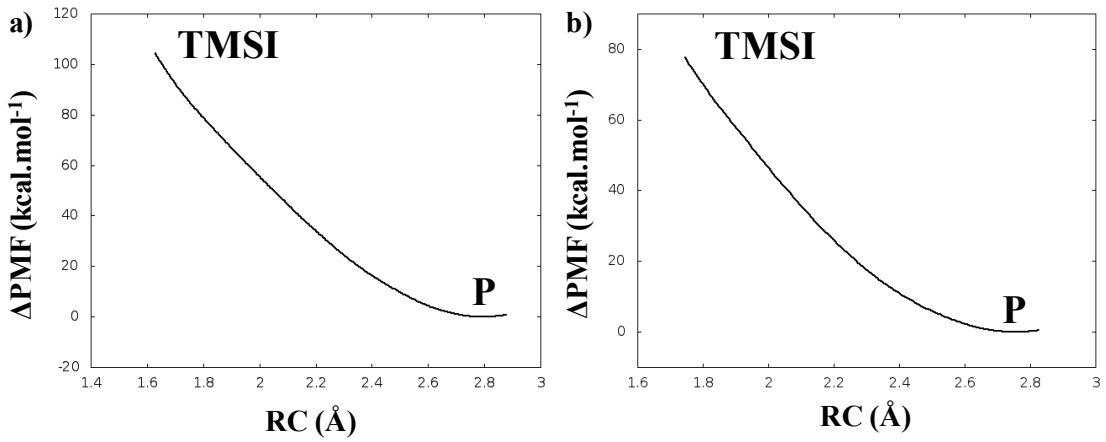

Figure S5. PMFs for the formation of TMSI intermediate from products, computed at AM1d/MM level: a) Bz-Tyr-Ala-CH_2F inhibitor, and b) Bz-Tyr-Ala-CH_2Cl inhibitor. RC corresponds to the distance d(SG-CT).

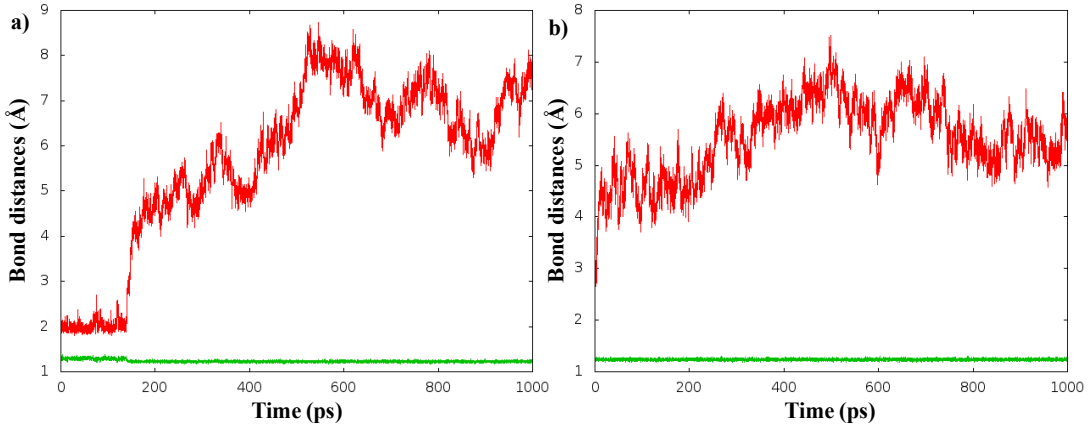

Figure S6. Time dependent evolution of selectd distances on the QM/MM MD simulation corresponding with the TH of the a) Bz-Tyr-Ala-CH_2F inhibitor and b) Bz-Tyr-Ala-CH_2Cl inhibitor. The green line corresponds to the OT-CT distances, and the red line with the SG-CT distance.

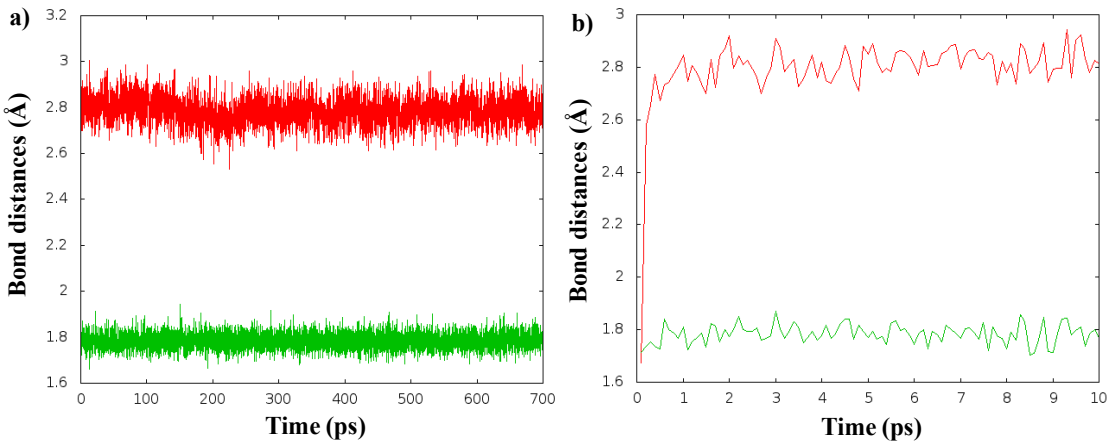

Figure S7. Time dependent evolution of selectd distances on the QM/MM MD simulation corresponding with the TMSI of the Bz-Tyr-Ala-CH_2F inhibitor. a) 700 ps of MD. b) first 10 ps of the 700 ps MD. The green line corresponds with the SG-CM distances, and the red line with the SG-CT distance.

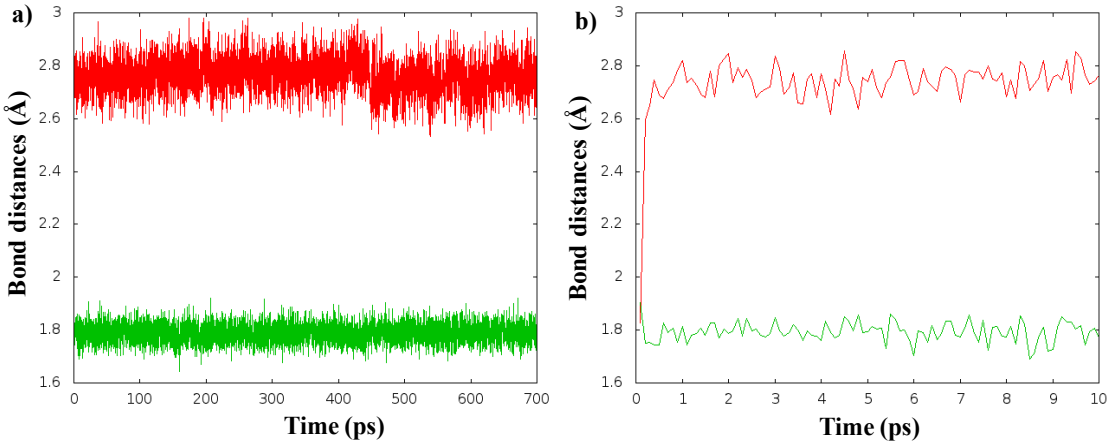

Figure S8. Time dependent evolution of selectd distances on the QM/MM MD simulation corresponding with the TMSI of the Bz-Tyr-Ala-CH_2Cl inhibitor. a) 700 ps of MD. b) first 10 ps of the 700 ps MD. The green line corresponds with the SG-CM distances, and the red line with the SG-CT distance.

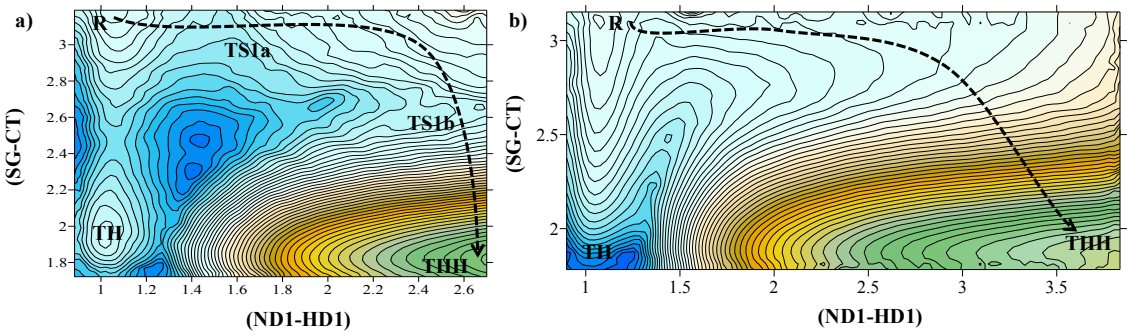

Figure S9. 2D-PMFs for the formation of protonated thiohemiketal intermediate, THH, from reactants, computed at AM1d/MM level: a) Bz-Tyr-Ala-CH_2F inhibitor, and b) Bz-Tyr-Ala-CH_2Cl inhibitor. Distances are in Å and iso-energetic lines are displayed every 1.0 kcal·mol^{-1}.

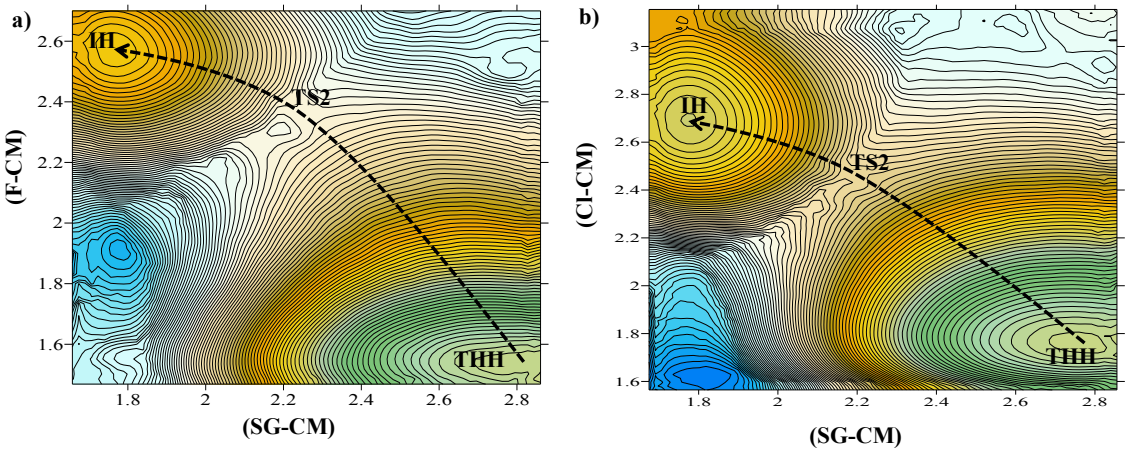

Figure S10. 2D-PMFs for the transformation from protonated thiohemiketal, THH to intermediate IH, computed at AM1d/MM level: a) Bz-Tyr-Ala-CH_2F inhibitor, and b) Bz-Tyr-Ala-CH_2Cl inhibitor. Distances are in Å and iso-energetic lines are displayed every 1.0 kcal·mol^{-1}.

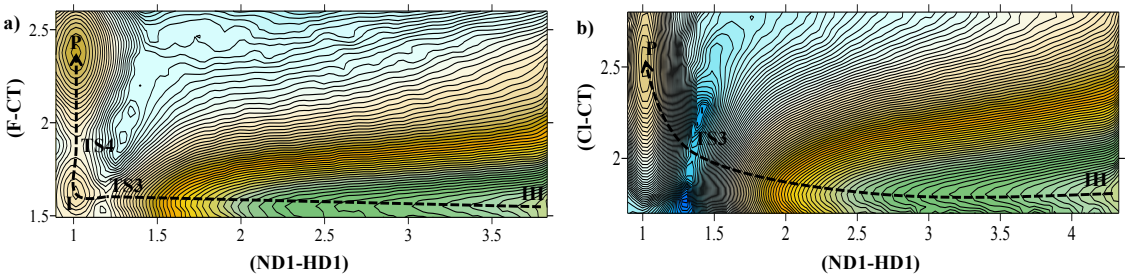

Figure S11. 2D-PMFs for the formation of products from IH intermediate computed at AM1d/MM level: a) Bz-Tyr-Ala-CH_2F inhibitor, and b) Bz-Tyr-Ala-CH_2Cl inhibitor. Distances are in Å and iso-energetic lines are displayed every 1.0 kcal·mol^{-1}.

Table S1. Averaged distances for key states located along the inhibition of cruzain by PHK, through a mechanism III. Results were obtained from 200 ps of AM1d/MM MD simulations on the stationary points taking from the M06-2X/6-31+G(d,p)/MM free energy profiles (in Å). Interatomic distances associated to the RCs were constrained during the simulations.

a) PFK.

d (Å)	R	RH	TS1	THH	TS2	IH	TS3	I	TS4	P
SG-CM	4.23±0.08	4.59±0.04	3.17±0.10	2.85±0.06	2.27±0.03	1.76±0.02	1.77±0.03	1.78±0.03	1.80±0.04	1.80±0.03
SG-CT	3.07±0.03	3.07±0.03	2.46±0.03	1.83±0.02	1.84±0.04	2.83±0.06	2.81±0.06	2.63±0.04	2.77±0.05	2.81±0.06
F-CM	1.55±0.02	1.56±0.03	1.54±0.02	1.54±0.02	2.23±0.03	2.57±0.02	2.55±0.04	2.51±0.04	2.70±0.05	2.81±0.08
F-CT	2.53±0.04	2.51±0.03	2.32±0.05	2.43±0.03	2.50±0.04	1.56±0.02	1.58±0.02	1.64±0.02	1.94±0.03	2.40±0.02
OT-CT	1.23±0.02	1.35±0.02	1.32±0.02	1.42±0.02	1.41±0.03	1.44±0.03	1.42±0.02	1.33±0.02	1.28±0.02	1.24±0.02
OT- HD1(His159)	4.09±0.29	1.03±0.03	1.01±0.03	0.98±0.03	0.98±0.03	0.97±0.03	1.09±0.04	4.65±0.22	5.77±0.40	4.97±0.54
ND1- HD1(His159)	1.03±0.02	1.73±0.03	1.93±0.03	3.02±0.03	7.13±0.63	3.26±0.55	1.32±0.03	1.00±0.02	1.00±0.02	1.00±0.02
F-HD1(His159)	6.27±0.17	4.44±0.07	3.45±0.17	3.31±0.25	2.91±0.52	2.69±0.13	2.52±0.06	3.05±0.13	6.46±0.35	6.33±0.36
F-H1	2.87±0.14	3.63±0.13	4.76±0.08	3.26±0.19	3.05±0.14	4.82±0.08	4.85±0.07	6.77±0.15	3.02±0.12	3.03±0.25
F-H2	2.87±0.09	2.97±0.13	3.08±0.18	3.04±0.18	3.07±0.11	3.33±0.20	3.35±0.13	2.71±0.10	3.20±0.11	2.76±0.22
SG-H(Trp26)	2.28±0.38	1.53±0.11	1.58±0.11	3.31±0.34	4.03±0.20	3.80±0.30	3.81±0.27	3.02±0.28	3.34±0.71	3.11±0.34
SG-HD1(His159)	1.97±0.10	2.68±0.14	3.13±0.15	2.85±0.13	3.16±0.21	3.55±0.41	3.71±0.14	5.98±0.14	4.12±0.44	3.67±0.65
SG-H(Gly160)	3.39±0.37	2.60±0.22	3.27±0.51	2.87±0.50	5.43±0.48	5.83±1.22	5.66±0.20	4.41±0.41	3.06±0.73	2.80±0.40
O1-H(Gly66)	6.09±0.18	5.66±0.17	4.01±0.30	5.85±0.45	6.58±0.32	4.16±0.37	4.28±0.36	2.85±0.30	6.60±0.27	5.07±0.62
O1-H(Trp26)	3.62±0.23	3.57±0.12	3.60±0.37	4.34±0.83	3.53±0.61	2.09±0.68	1.72±0.42	3.81±0.40	4.72±1.09	1.96±1.14
O2-H(Gly66)	2.37±0.25	4.82±0.32	3.65±0.56	5.45±0.37	5.55±0.36	3.95±0.37	3.91±0.24	4.34±0.34	5.79±0.43	2.82±0.61
O3-H(Gly66)	4.18±0.33	2.77±0.32	3.54±0.42	3.61±0.56	3.50±0.42	3.24±0.42	2.89±0.45	3.36±0.34	3.70±0.44	4.73±0.44
N1-H(Gly66)	4.20±0.27	3.43±0.27	3.42±0.48	3.77±0.25	4.16±0.34	2.66±0.30	2.71±0.24	2.86±0.27	4.40±0.40	3.81±0.30
N2-H(Trp26)	2.24±0.25	2.74±0.16	4.11±0.29	3.03±0.63	2.08±0.61	3.31±0.37	3.34±0.25	4.57±0.26	3.15±0.96	2.80±0.30
H1-N(Gly66)	6.03±0.29	3.79±0.24	4.30±0.70	3.73±0.22	3.99±0.25	3.36±0.41	3.43±0.25	3.12±0.24	4.33±0.40	5.16±1.04
H2-O(Gly66)	4.89±0.41	1.92±0.14	2.73±0.58	2.18±0.35	2.20±0.29	2.22±0.22	2.14±0.20	1.98±0.20	2.44±0.42	3.76±0.88
H2-O(Asp158)	6.27±0.25	5.68±0.28	6.31±0.31	5.75±0.18	5.89±0.21	5.66±0.57	4.66±0.30	1.90±0.16	6.31±0.23	5.98±0.18
H2-N(Trp26)	3.53±0.26	3.83±0.20	5.88±0.45	4.06±0.31	3.32±0.41	5.00±0.41	5.09±0.20	5.77±0.23	3.96±0.46	4.52±0.36

b) PClK.

d (Å)	R	THH	TS2	IH	TS3	P
SG-CM	3.57 ± 0.21	2.78 ± 0.06	1.99±0.02	1.77±0.02	1.79±0.04	1.79±0.03
SG-CT	3.00 ± 0.03	1.88 ± 0.02	2.90±0.07	2.77±0.05	2.81±0.05	2.77±0.06
Cl-CM	1.77 ± 0.03	1.77 ± 0.03	2.55±0.02	2.69±0.02	2.77±0.06	2.96±0.13
Cl-CT	2.71 ± 0.05	2.75 ± 0.05	1.80±0.04	1.81±0.04	1.94±0.02	2.57±0.03
OT-CT	1.24 ± 0.02	1.42 ± 0.02	1.41±0.03	1.41±0.02	1.37±0.02	1.24±0.02
OT- HD1 (His159)	3.74 ± 0.36	0.97 ± 0.03	0.98±0.03	0.97±0.03	1.10±0.04	4.86±0.34
ND1- HD1 (His159)	1.03 ± 0.02	3.70 ± 0.03	6.14±0.42	5.86±0.68	1.45±0.03	1.00±0.02
Cl-HD1 (His159)	3.93 ± 0.35	2.58 ± 0.18	3.05±0.22	2.87±0.20	2.81±0.11	4.66±0.51
SG-H (Trp26)	2.99 ± 0.23	3.16 ± 0.19	2.66±0.25	2.93±0.27	3.51±0.44	3.00±0.24
SG-H (His159)	1.93 ± 0.09	2.93 ± 0.11	3.91±0.23	3.81±0.23	3.15±0.20	3.93±0.52
SG-H (Gly160)	2.60 ± 0.21	3.00 ± 0.26	3.29±0.33	3.40±0.55	3.74±0.59	2.88±0.32
OT-H (Cys25)	3.48 ± 0.36	2.51 ± 0.23	4.10±0.91	2.89±0.34	4.55±0.37	3.07±0.56
OT-H (Gln19)	3.90 ± 0.63	2.65 ± 0.33	4.72±0.50	4.00±0.62	5.48±0.56	3.51±0.91
O1-H (Gly66)	3.50 ± 0.39	3.20 ± 0.35	3.75±0.65	3.27±0.58	3.06±0.40	3.72±0.42
O1-H (Trp26)	1.71 ± 0.24	1.66 ± 0.24	1.76±0.36	2.03±0.62	1.90±0.39	1.53±0.24
O3-H (Gly66)	2.95 ± 0.47	3.21 ± 0.38	2.84±0.52	3.21±0.52	3.54±0.40	3.26±0.64
N1-H (Gly66)	2.71 ± 0.22	2.47 ± 0.21	2.96±0.29	2.88±0.30	2.48±0.24	2.95±0.26
H1-O (Gly66)	1.94 ± 0.15	1.92 ± 0.15	1.93±0.19	1.96±0.18	2.00±0.18	1.97±0.19
H1-N (Gly66)	3.23 ± 0.24	3.09 ± 0.22	3.47±0.28	3.40±0.30	3.13±0.24	3.51±0.28
H2-O (Asp158)	2.92 ± 0.33	3.10 ± 0.29	3.44±0.57	3.21±0.35	4.85±0.37	3.65±0.46