Supporting Information for

Atomistic-level description of the covalent inhibition of SARS-CoV-2 papain-like protease

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1. RMSD simulation analysis
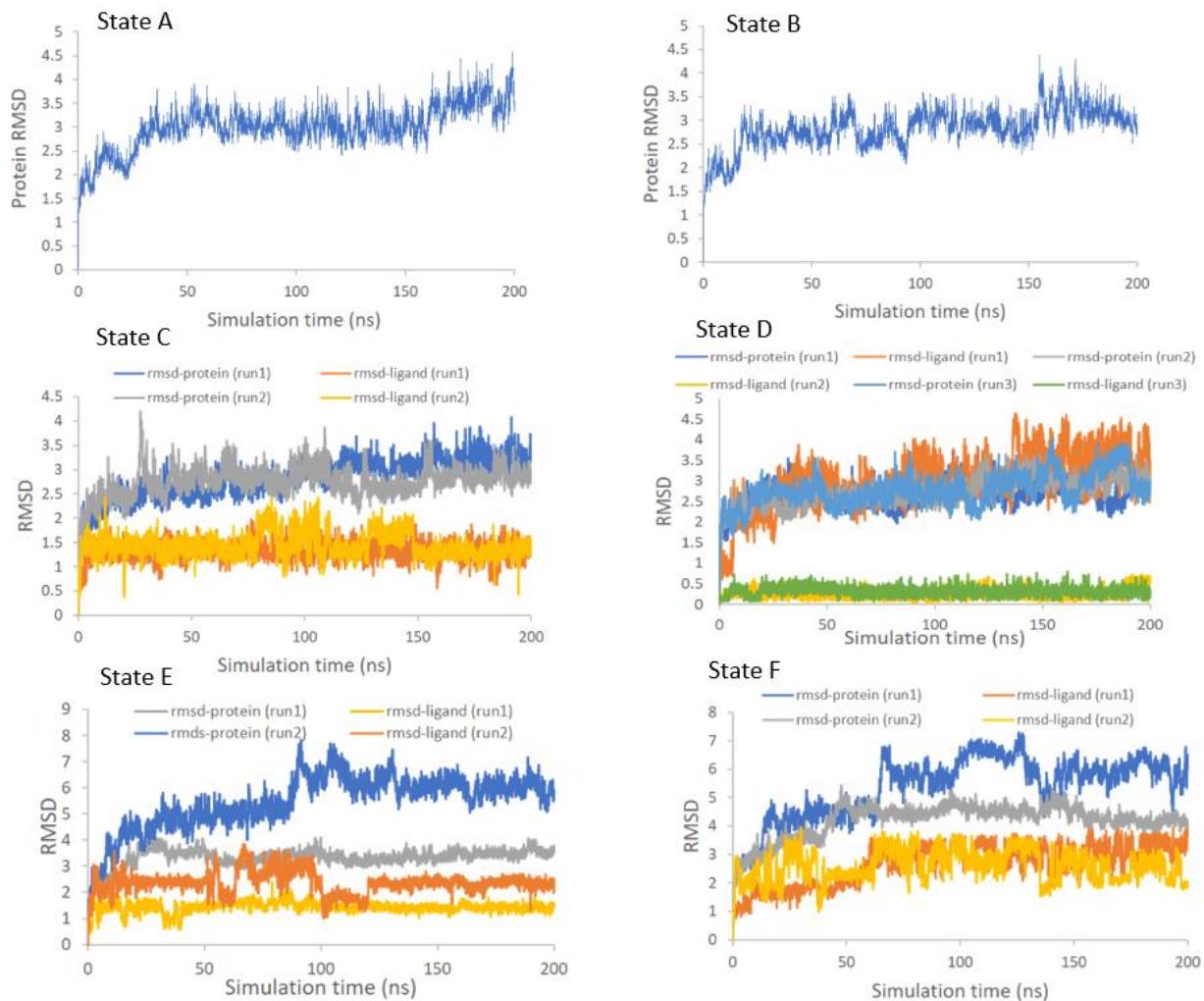
Figure S1. Protein RMSD analysis for each mechanism state under study (see Figure 1 for state nomenclature). For states C-F also the ligand RMSD analysis is depicted.

2. MMPBSA results

Table S1. MMPBSA results.

Step	Run	ΔG (kcal/mol)	TΔS (kcal/mol)	ΔH (kcal/mol)
A → C	1	-16.8722 ± 6.1415	-31.1789 ± 3.987	-48,0511
	2	-14.3902 ± 7.0834	-30.5637 ± 4.7092	-44,9539
B → D	2	5.6311 ± 8.9885	-28.7669 ± 5.1475	-23,1358
	3	-2.3268 ± 7.9644	-27.521 ± 4.2873	-29,8478

3. Time series data for the corresponding histograms
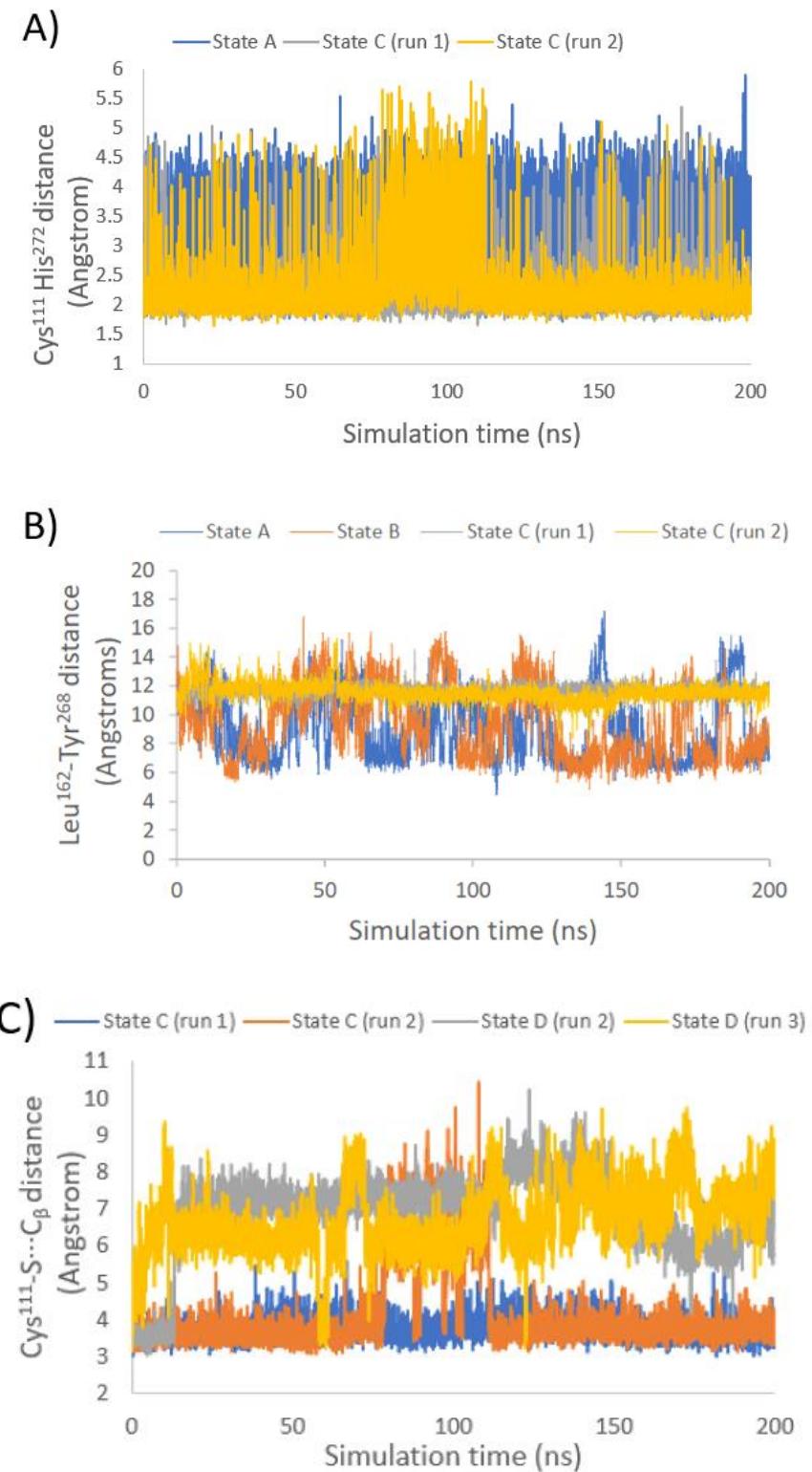
**Figure S2.** Time series data corresponding to histograms of A) figure 3B, B) figure 4C and C) figure 5B.

4. Ligand departure from the protease pocket in state D
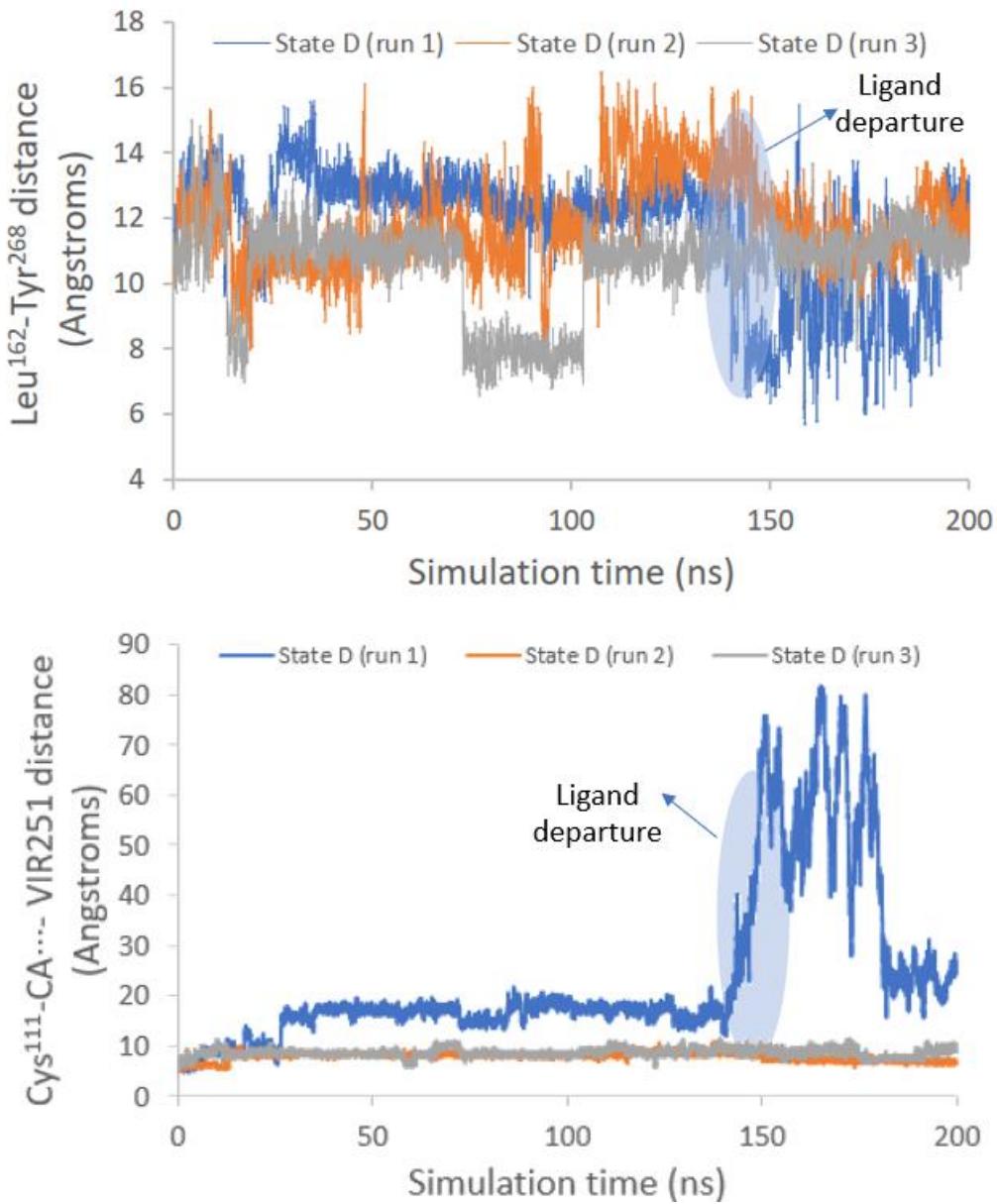
**Figure S3.** A) BL2 conformation measured through the Leu^{162}-Tyr^{268} distance and B) Cys^{111}-VIR251 distance along the simulation time, showing both an abrupt change of their values when the ligand moves out from the protease pocket.

5. Analysis of the thiol group conformations for Cys[111]
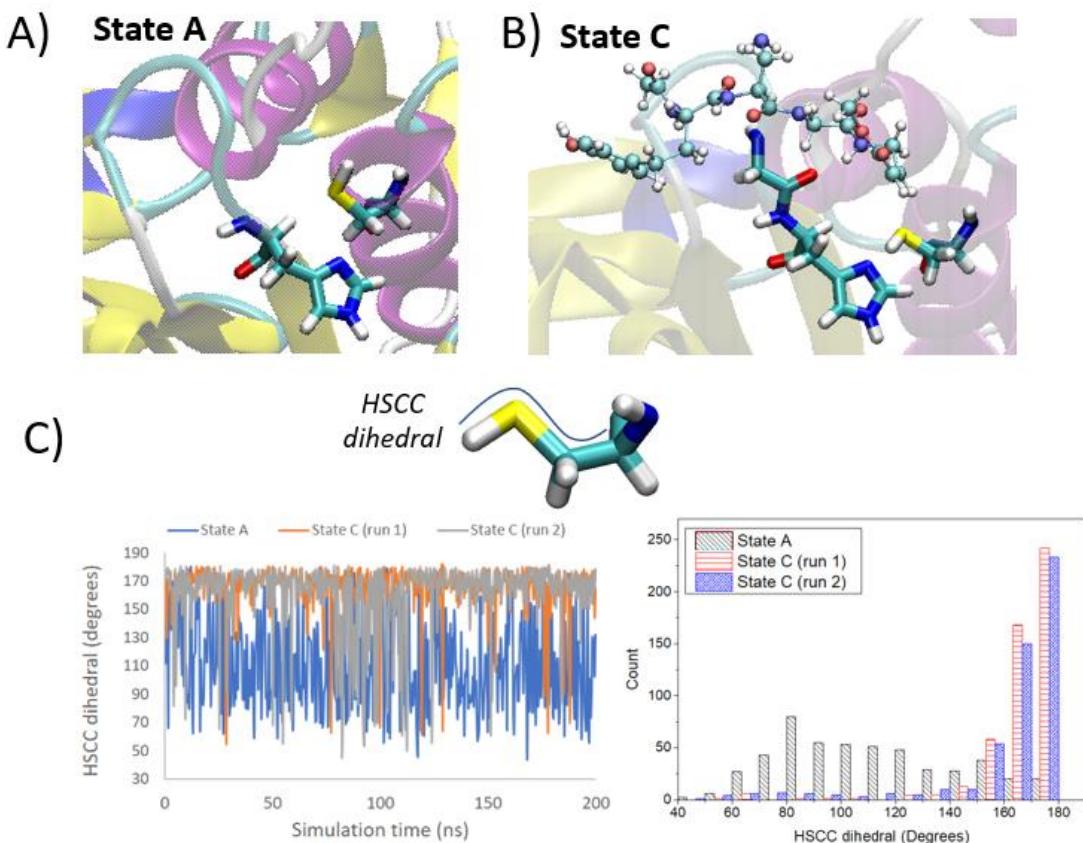
Figure S4. A) Representative snapshot of state A showing a particular conformation of Cys[111], pointing outwards His[272] and hence, hampering the hydrogen-bond interaction. B) Representative snapshot of state B, with the thiol group pointing towards the His[272]. Ligand is depicted in CPK representation showing its position on top of Cys[111] hampering it's the S-C free rotation. C) Time series and histogram of the HSCC dihedral angle (defined in the figure) showing the conformations populated during the MD simulations of states A and C.

6. QM/MM energy barrier for Cys[111] deprotonation
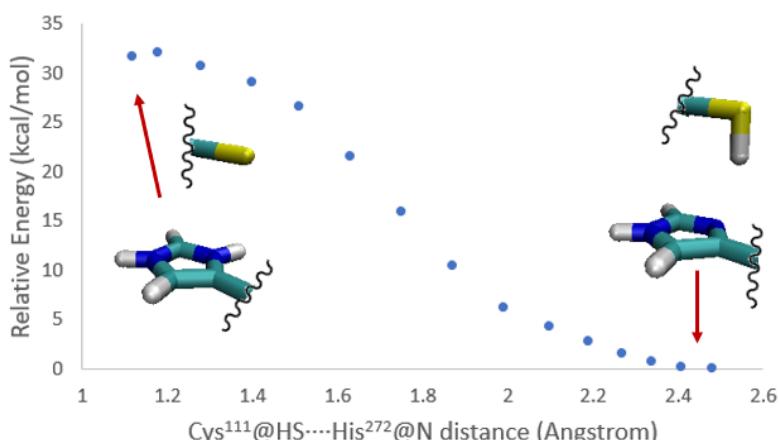
Figure S5. Energy barrier for the proton transfer process, from Cys[111] to His[272] computed for state A at the QM/MM level.

7. Hydrogen-bond interaction analysis

Table S2. Fraction of hydrogen-bond interactions found along the simulation time of each MD trajectory between the protease residues and the ligand.

Residue	State C (Run 1)	State C (Run 2)	State D (Run 2)	State D (Run 3)	State E (Run 1)	State E (Run 2)	State F (Run 1)	State F (Run 2)
Tyr 264	0.9158	0.6856	0.3016	0.6876	0.8654		0.8650	0.3174
Gly 271	0.7694	0.7776	0.1580	0.1990	0.7966	0.6394	0.6746	0.6798
Gly 163@O	0.6020	0.6344	0.5936	0.5630	0.7826	0.8106	0.7754	0.7598
Gly 163@H	0.3310	0.3216	0.5048	0.1560	0.5558	0.4406	0.4582	0.5256
Tyr 268	0.5048	0.4120	0.1358	0.3190	0.4722		0.1278	0.1598
Arg 166	0.3304	0.3256	<0.100	0.1504	0.4300	0.3868		
Tyr 273						0.6078		

8. BL2 loop conformation analysis for state D
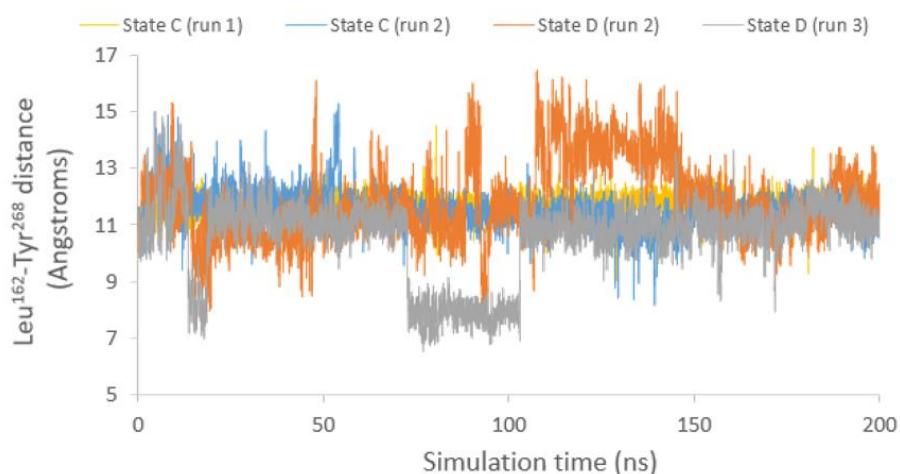
Figure S6. Leu^{162}-Tyr^{268} distance along the MD simulations of states C and D, showing a less stable conformation of the BL2 loop for state D.

9. Conformations of the ligand CH$_2$-CO-NH group
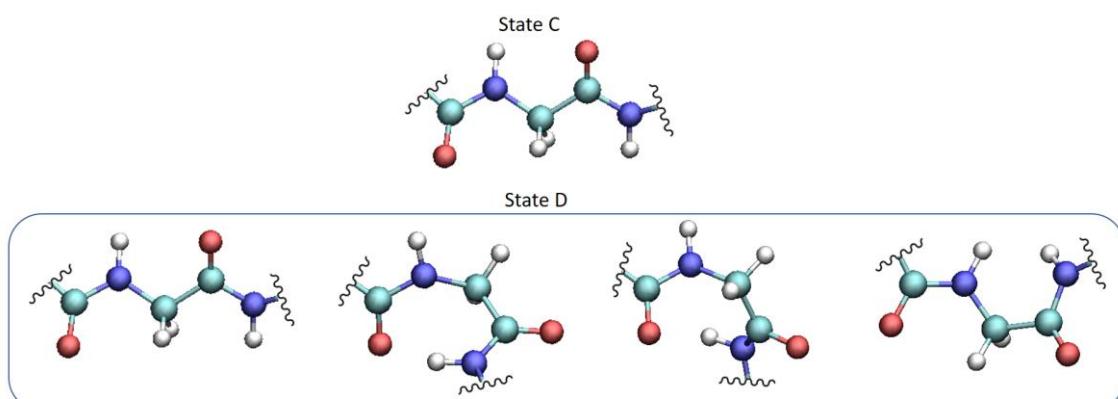
Figure S7. Schematic representations of the most populated conformation for the CH$_2$-CO-NH group of the ligand for states C and D.

10. Distance analysis for state D between the $Cys^{111}@S^-$ and $Vir251@C_\beta$ or $His^{272}@H^+$
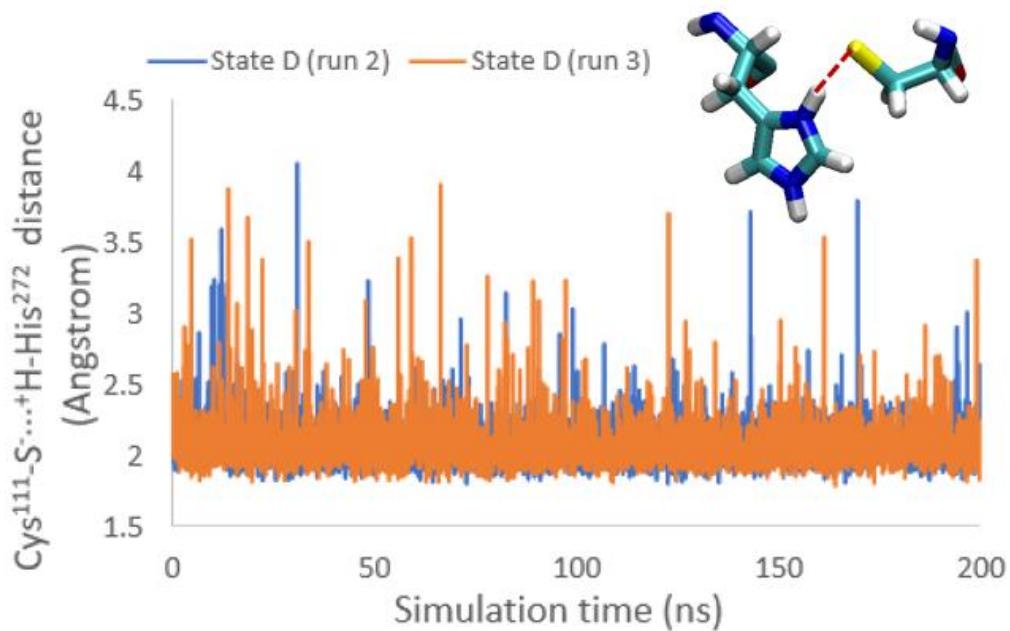
Figure S8. Distance between the negatively charged S atom of Cys^{111} and the transferred proton to His^{272} along the simulation time for the two independent simulations of state D.
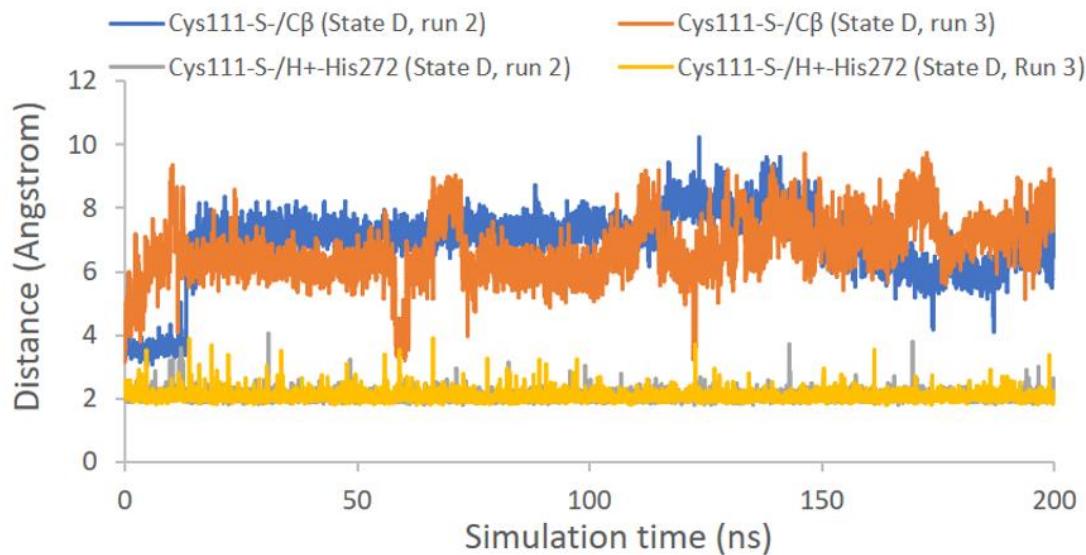
Figure S9. Cys^{111}-S$^-$⋯C_β-VIR251 and Cys^{111}-S$^-$⋯$^+$H-His^{272} distance along the MD simulations of state D.

11. Hydrogen-bond interaction analysis between the vinyl methyl ester group and water molecules or protease side chains

Table S3. Fraction of hydrogen-bond interactions between the oxygen of the carbonyl group or oxygen of the methoxy group conforming the vinyl methyl ester group with water molecules or protease residues along the MD simulation.

Residue	State C (Run 1)	State C (Run 2)	State D (Run 2)	State D (Run 3)	State E (Run 1)	State E (Run 2)
Carbonyl oxygen (C=O)	Water (1.2170)	Water (1.2206)	Water (0.7406) GLY[160]@H (0.3836)	Water (1.1598)	Water (1.8496)	Water (1.7736)
Methoxy oxygen (OMe)					Water (0.4502)	Water (0.5310)

12. Ligand force field details

Table S4. Atom type, xyz coordinates of the optimized geometry and RESP charges for VIR251 state C and D.

| Name	X	Y	Z	Atom type	Charge
C1	-6.4700	-1.9210	-0.2790	c	0.880559
O1	-6.6230	-1.0080	-1.1040	o	-0.664432
C2	-7.4510	-3.0730	-0.1840	c3	-0.486499
N1	-5.4270	-1.9560	0.5900	n	-0.809988
C3	-4.3860	-0.9340	0.6550	c3	0.242707
C4	-3.0300	-1.6600	0.6170	c	0.483729
C5	-4.5310	-0.0850	1.9360	c3	0.008453
C6	-3.5510	1.1020	2.0470	c3	-0.132125
C7	-3.7470	2.1890	1.0060	ca	0.058878
C8	-2.7860	2.4390	0.0140	ca	-0.178927
C9	-2.9620	3.4440	-0.9400	ca	-0.331208
C10	-4.1190	4.2270	-0.9150	ca	0.430254
C11	-5.0930	4.0010	0.0650	ca	-0.331208
C12	-4.8990	2.9920	1.0110	ca	-0.178927
O2	-4.2460	5.2060	-1.8720	oh	-0.572924
O3	-2.7470	-2.4990	1.4830	o	-0.587626
N2	-2.2090	-1.3220	-0.4010	n	-0.396462
C13	-0.8790	-1.8790	-0.6020	c3	-0.012751
C14	0.1140	-0.7160	-0.7440	c	0.450022
O4	-0.1600	0.2640	-1.4520	o	-0.544209
C15	-0.8020	-2.7680	-1.8650	c3	0.256483
N3	-1.6720	-3.9400	-1.7430	n3	-0.967320

N4	1.2840	-0.8470	-0.0920	n	-0.288379
C16	2.3360	0.1450	-0.1740	c3	-0.329888
C17	3.4940	-0.2830	0.7300	c	0.752807
O5	3.4430	-1.3280	1.3890	o	-0.554363
N5	4.5440	0.5670	0.7460	n	-0.763331
C18	5.7520	0.3510	1.5410	c3	0.316863
C19	6.9170	-0.0610	0.6840	c2	-0.003945
C20	8.0930	0.5810	0.6580	ce	-0.588760
C21	9.2090	0.1160	-0.1960	c	0.977035
O6	10.2760	0.9340	-0.0790	os	-0.437097
O7	9.1970	-0.8690	-0.9200	o	-0.626795
C22	11.4410	0.5960	-0.8630	c3	-0.039803
H1	4.5100	1.3910	0.1590	hn	0.385284
H2	1.4720	-1.6420	0.5100	hn	0.223695
H3	-1.2620	-4.6250	-1.1120	hn	0.368908
H4	-1.7640	-4.3970	-2.6460	hn	0.368908
H5	-5.3510	-2.7280	1.2390	hn	0.394975
H6	-2.5050	-0.6120	-1.0590	hn	0.296740
H7	-5.0800	5.6830	-1.7520	ho	0.386050
H8	-8.4410	-2.6720	0.0520	hc	0.131871
H9	-7.1780	-3.8140	0.5710	hc	0.131871
H10	-7.5140	-3.5630	-1.1590	hc	0.131871
H11	-1.8780	1.8420	-0.0200	ha	0.162798
H12	-2.2090	3.6270	-1.7010	ha	0.187063
H13	-5.9920	4.6110	0.0920	ha	0.187063
H14	-5.6620	2.8360	1.7700	ha	0.162798
H15	0.2560	-3.0220	-2.0320	h1	0.018087
H16	-1.1340	-2.1780	-2.7250	h1	0.018087
H17	1.9650	1.1270	0.1400	h1	0.164031
H18	2.6930	0.2490	-1.2060	h1	0.164031
H19	8.2730	1.4670	1.2600	ha	0.207645
H20	12.1650	1.3840	-0.6580	h1	0.092731
H21	11.1890	0.5720	-1.9260	h1	0.092731
H22	11.8330	-0.3760	-0.5550	h1	0.092731
H23	-4.5140	-0.3030	-0.2270	h1	0.094168
H24	-4.3980	-0.7440	2.8020	hc	0.014723
H25	-5.5630	0.2820	1.9660	hc	0.014723
H26	-2.5180	0.7360	2.0030	hc	0.055631
H27	-3.6760	1.5330	3.0480	hc	0.055631
H28	5.5200	-0.4420	2.2580	h1	0.035621
H29	5.9800	1.2630	2.1010	h1	0.035621
H30	6.7740	-0.9390	0.0560	ha	0.135263
H31	-0.6490	-2.4800	0.2820	h1	0.157827

Table S5. Atom type, xyz coordinates of the optimized geometry and RESP charges for VIR251 state E.

Name	X	Y	Z	Atom type	Charge
N1	-6.9380	-1.5930	0.2770	N	-0.395868
C1	-6.9770	-2.0750	-1.1140	CT	-0.047432
H1	-8.0070	-2.3520	-1.3950	H1	0.081191
C2	-6.5640	-0.9100	-2.0680	CT	-0.152196
S1	-4.9380	-0.0640	-1.7480	S	-0.405786
C3	-6.1540	-3.3760	-1.3530	C	0.625516
O1	-6.7290	-4.4140	-1.7390	O	-0.632980
C4	7.6370	-1.4410	1.6000	C	0.772756
O2	8.0690	-0.3100	1.4160	O	-0.628271
C5	8.4760	-2.5280	2.2510	CT	-0.498254
N2	6.3670	-1.7980	1.2760	N	-0.579361
C6	5.3630	-0.9010	0.6600	CT	0.200970
C7	4.2310	-0.6150	1.6470	C	0.525652
C8	4.7720	-1.4790	-0.6630	CT	0.000758
C9	5.8440	-1.8940	-1.7120	CT	-0.104643
C10	6.8100	-0.7740	-2.1080	CA	0.104245
C11	6.3340	0.3900	-2.7370	CA	-0.237827
C12	7.2270	1.4010	-3.1220	CA	-0.292525
C13	8.6010	1.2550	-2.8850	CA	0.422208
C14	9.0810	0.0940	-2.2600	CA	-0.292525
C15	8.1880	-0.9150	-1.8760	CA	-0.237827
O3	9.4690	2.2400	-3.2730	OH	-0.582249
O4	3.9100	-1.4000	2.5330	O	-0.571752
N3	3.5220	0.5170	1.4000	N	-0.608273
C16	2.4350	1.0380	2.2560	CT	-0.013109
C17	1.2770	1.5200	1.3870	C	0.669608
O5	1.1720	2.6800	1.0010	O	-0.611548
C18	2.9280	2.1630	3.2060	CT	0.276963
N4	3.9500	1.6640	4.1590	NT	-0.944886
N5	0.4550	0.5360	0.9360	N	-0.631801
C19	-0.6750	0.7370	0.0050	CT	-0.057608
C20	-2.0280	0.5510	0.6810	C	0.705872
O6	-2.2540	-0.3930	1.4270	O	-0.633145
N6	-2.9980	1.4330	0.3040	N	-0.534075
C21	-4.4080	1.2660	0.6740	CT	-0.009154
C22	-5.3560	1.3530	-0.5580	CT	0.561084
C23	-5.3350	2.7100	-1.2220	CM	-1.169581
C24	-5.9700	3.7570	-0.5540	C	0.964388
O7	-5.7180	5.0400	-1.1140	OS	-0.477979
O8	-6.7170	3.6860	0.4760	O	-0.789536
C25	-6.2080	6.1630	-0.3660	CT	-0.005846
H2	-2.7670	2.2250	-0.2940	H	0.323893

Name	X	Y	Z	Atom type	Charge
H3	0.6210	-0.4070	1.2600	H	0.348382
H4	3.5620	0.9270	4.7550	H	0.357922
H5	4.2710	2.4180	4.7790	H	0.357922
H6	6.0670	-2.7400	1.4850	H	0.316155
H7	3.8100	1.1110	0.6250	H	0.369668
H8	10.3820	1.9940	-3.0410	HO	0.389405
H9	9.4240	-2.6470	1.6980	HC	0.134055
H10	7.9600	-3.4970	2.2370	HC	0.134055
H11	8.7000	-2.2650	3.2810	HC	0.134055
H12	5.2650	0.5200	-2.9390	HA	0.186364
H13	6.8530	2.3100	-3.6120	HA	0.179764
H14	10.1560	-0.0190	-2.0830	HA	0.179764
H15	8.5770	-1.8150	-1.4010	HA	0.186364
H16	2.0880	2.5630	3.7790	H1	0.014315
H17	3.3750	2.9800	2.6150	H1	0.014315
H18	-0.6130	0.0020	-0.7960	H1	0.108304
H19	-0.6340	1.7470	-0.4340	H1	0.108304
H20	-5.6790	7.0430	-0.7550	H1	0.054318
H21	-6.0010	6.0520	0.7100	H1	0.054318
H22	-7.2940	6.3000	-0.4990	H1	0.054318
H23	5.8400	0.0680	0.4310	H1	0.091267
H24	4.1020	-0.7280	-1.1140	HC	0.020257
H25	4.1560	-2.3530	-0.4320	HC	0.020257
H26	5.3250	-2.2410	-2.6220	HC	0.033246
H27	6.4070	-2.7530	-1.3370	HC	0.033246
H28	-4.7120	2.0720	1.3620	H1	0.064090
H29	-4.4900	0.3080	1.2040	H1	0.064090
H30	2.0430	0.2180	2.8740	H1	0.138059
H31	-7.3560	-0.1470	-2.0810	H1	0.132671
H32	-6.4830	-1.3140	-3.0880	H1	0.132671
H33	-6.3720	1.1630	-0.1710	H1	0.060992
H38	-6.6460	-0.6280	0.4050	H	0.207071
H42	-4.6500	2.9090	-2.0470	HA	0.230939

Table S6. Atom type, xyz coordinates of the optimized geometry and RESP charges for VIR251 state F.

Name	X	Y	Z	Atom type	Charge
N1	-6.9060	-1.6420	0.3390	N	-0.532183
C1	-6.9230	-2.1450	-1.0480	CT	0.051602
H1	-7.9450	-2.4440	-1.3360	H1	0.085178
C2	-6.5250	-0.9900	-2.0210	CT	-0.168901
S1	-4.8990	-0.1350	-1.7140	S	-0.280978
C3	-6.0690	-3.4280	-1.2700	C	0.588075
O1	-6.6190	-4.4730	-1.6810	O	-0.605077

C4	7.6270	-1.4040	1.6520	C	0.783638
O2	8.0660	-0.2850	1.4380	O	-0.619624
C5	8.4570	-2.4830	2.3350	CT	-0.536694
N2	6.3540	-1.7660	1.3280	N	-0.573136
C6	5.3600	-0.8790	0.6840	CT	0.188440
C7	4.2240	-0.5590	1.6580	C	0.528873
C8	4.7730	-1.4870	-0.6260	CT	-0.002599
C9	5.8490	-1.9360	-1.6610	CT	-0.103672
C10	6.8240	-0.8310	-2.0800	CA	0.101005
C11	6.3590	0.3200	-2.7420	CA	-0.234686
C12	7.2600	1.3160	-3.1470	CA	-0.296047
C13	8.6330	1.1660	-2.8990	CA	0.435855
C14	9.1030	0.0190	-2.2420	CA	-0.296047
C15	8.1980	-0.9750	-1.8310	CA	-0.234686
O3	9.5120	2.1410	-3.3000	OH	-0.590521
O4	3.8950	-1.3200	2.5660	O	-0.565310
N3	3.5210	0.5730	1.3750	N	-0.598755
C16	2.4340	1.1190	2.2150	CT	-0.022317
C17	1.2800	1.5870	1.3270	C	0.665286
O5	1.1800	2.7400	0.9140	O	-0.608917
C18	2.9330	2.2680	3.1400	CT	0.283176
N4	3.9440	1.7850	4.1120	NT	-0.941680
N5	0.4560	0.5980	0.8960	N	-0.596828
C19	-0.6670	0.7810	-0.0460	CT	-0.057821
C20	-2.0260	0.6200	0.6270	C	0.688906
O6	-2.2630	-0.3010	1.3970	O	-0.586121
N6	-2.9780	1.5120	0.1970	N	-0.542234
C21	-4.3910	1.4290	0.5780	CT	0.019361
C22	-5.3660	1.3220	-0.6280	CT	0.075540
C23	-5.4760	2.6230	-1.4640	CT	-0.611921
C24	-6.1200	3.7510	-0.6550	C	0.980876
O7	-5.5070	4.9380	-0.8570	OS	-0.451921
O8	-7.0880	3.6030	0.0800	O	-0.622342
C25	-6.0610	6.0850	-0.1460	CT	-0.041163
H2	-2.6740	2.3090	-0.3580	H	0.334897
H3	0.6210	-0.3480	1.2440	H	0.346424
H4	3.5490	1.0670	4.7210	H	0.364190
H5	4.2640	2.5560	4.7120	H	0.364190
H6	6.0520	-2.6990	1.5640	H	0.318191
H7	3.8280	1.1430	0.5930	H	0.374131
H8	10.4320	1.9030	-3.0550	HO	0.400920
H9	9.4160	-2.6130	1.7930	HC	0.148401
H10	7.9390	-3.4500	2.3440	HC	0.148401
H11	8.6820	-2.1880	3.3720	HC	0.148401
H12	5.3010	0.4460	-2.9510	HA	0.187440

H13	6.8980	2.2130	-3.6630	HA	0.183363
H14	10.1800	-0.1070	-2.0510	HA	0.183363
H15	8.5920	-1.8620	-1.3360	HA	0.187440
H16	2.0860	2.6840	3.6950	H1	0.012748
H17	3.3860	3.0640	2.5270	H1	0.012748
H18	-0.5990	0.0120	-0.8310	H1	0.110117
H19	-0.6110	1.7730	-0.5130	H1	0.110117
H20	-5.4070	6.9250	-0.4010	H1	0.100978
H21	-6.0490	5.9000	0.9380	H1	0.100978
H22	-7.0890	6.2790	-0.4800	H1	0.100978
H23	5.8500	0.0820	0.4350	H1	0.091801
H24	4.1180	-0.7470	-1.1000	HC	0.015572
H25	4.1600	-2.3550	-0.3740	HC	0.015572
H26	5.3440	-2.3060	-2.5600	HC	0.040874
H27	6.4160	-2.7900	-1.2540	HC	0.040874
H28	-4.6690	2.3120	1.1810	H1	0.092366
H29	-4.4940	0.5460	1.2220	H1	0.092366
H30	2.0370	0.3150	2.8510	H1	0.137176
H31	-7.3220	-0.2330	-2.0420	H1	0.142467
H32	-6.4440	-1.4050	-3.0350	H1	0.142467
H33	-6.3680	1.1310	-0.2140	H1	0.133942
H34	-6.1340	2.4420	-2.3300	HC	0.186709
H35	-4.5040	2.9450	-1.8630	HC	0.186709
H40	-6.7630	-0.6430	0.4530	H	0.289036