Supporting Information: Unveiling the Effect of Low pH on the SARS-CoV-2 Main Protease by Molecular Dynamics Simulations

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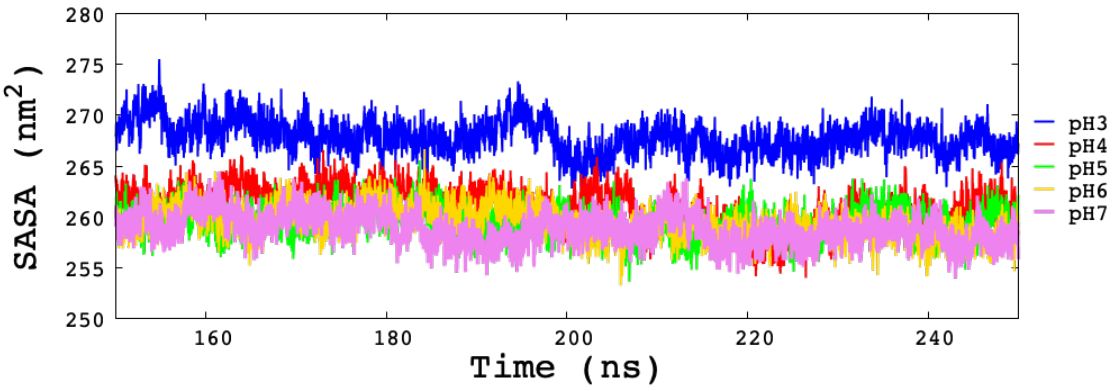
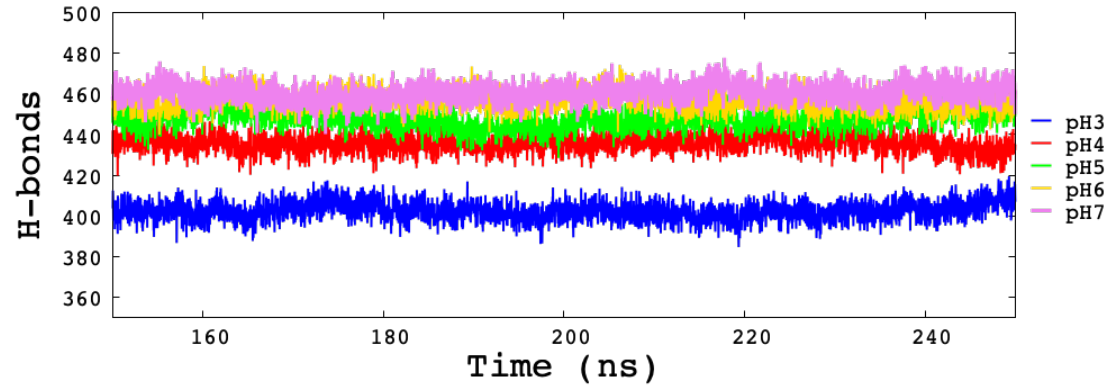


(a)



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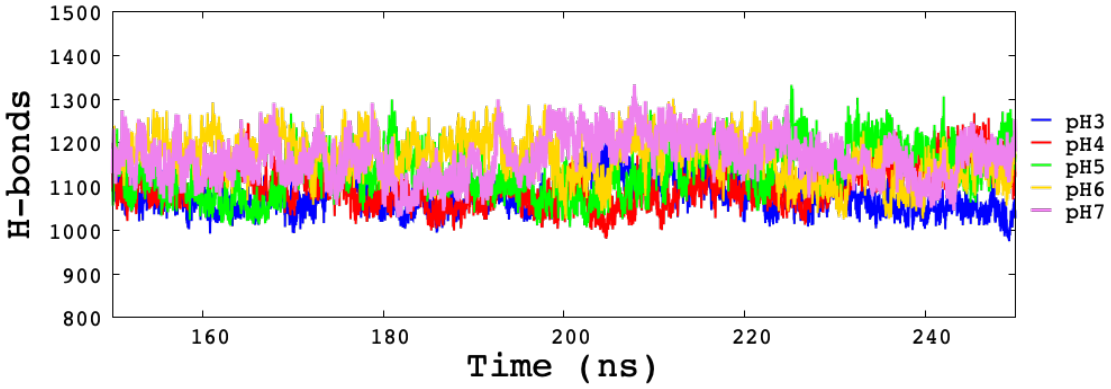
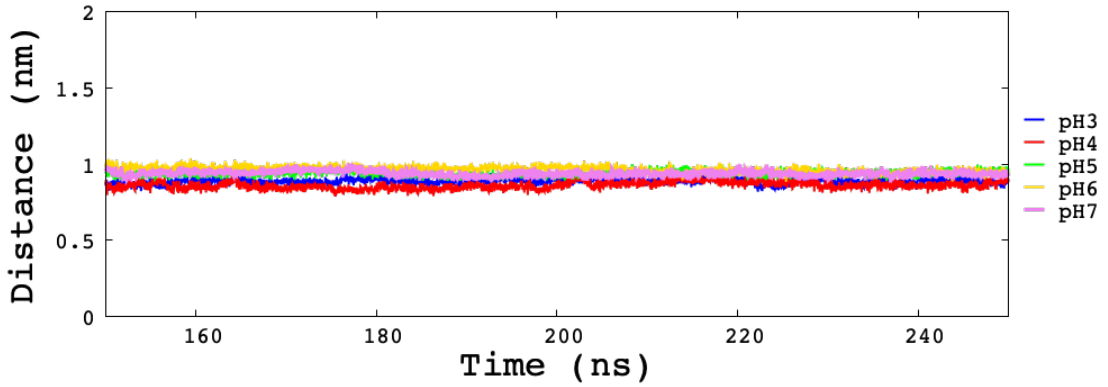
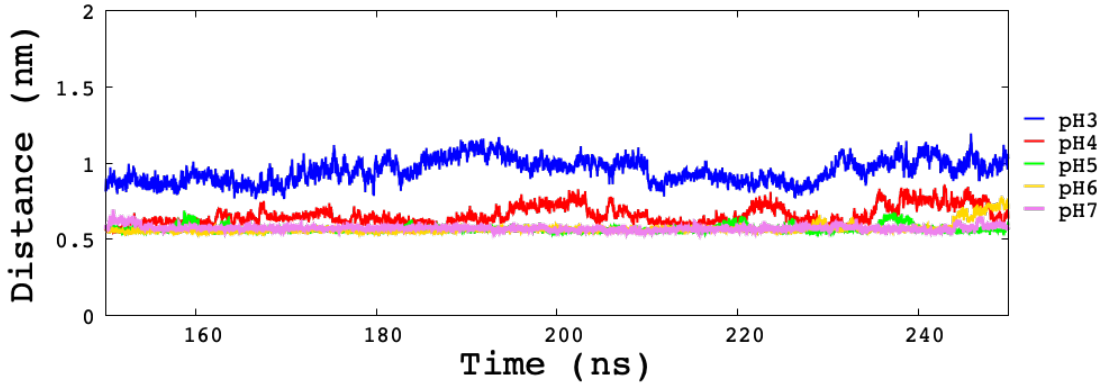


(c)

Figure S1. Plot of hydrogen bonds and SASA during the time of MD simulations at different pHs. (a) Solvent Accessible Surface Area. (b) Intramolecular Hydrogen bonds between protein-protein. (c) Intermolecular Hydrogen bonds between protein-waters.



(a)



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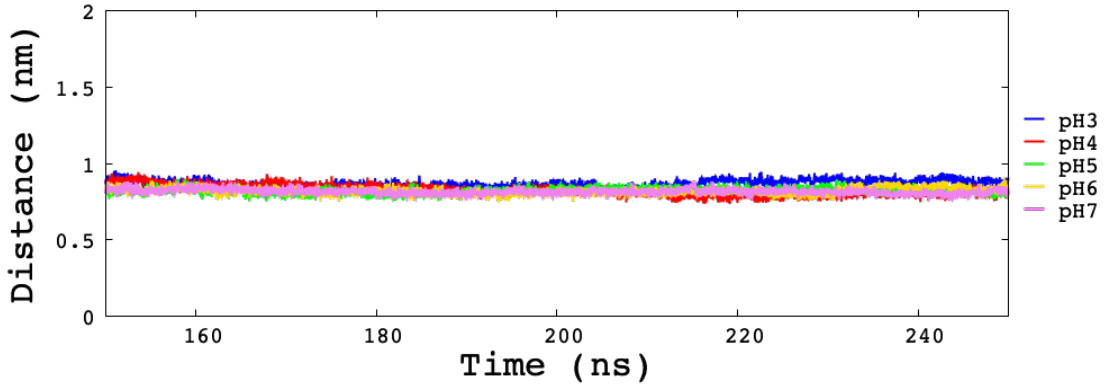

(c)

Figure S2. Distances between C-α atoms from residues of the active site (Cys145 and His41) and S1 pocket in Mpro. (a) Average distance Cys145-His41. (b) Average distance of Met49-Gln189. (c) Average distance of His172-Phe140.

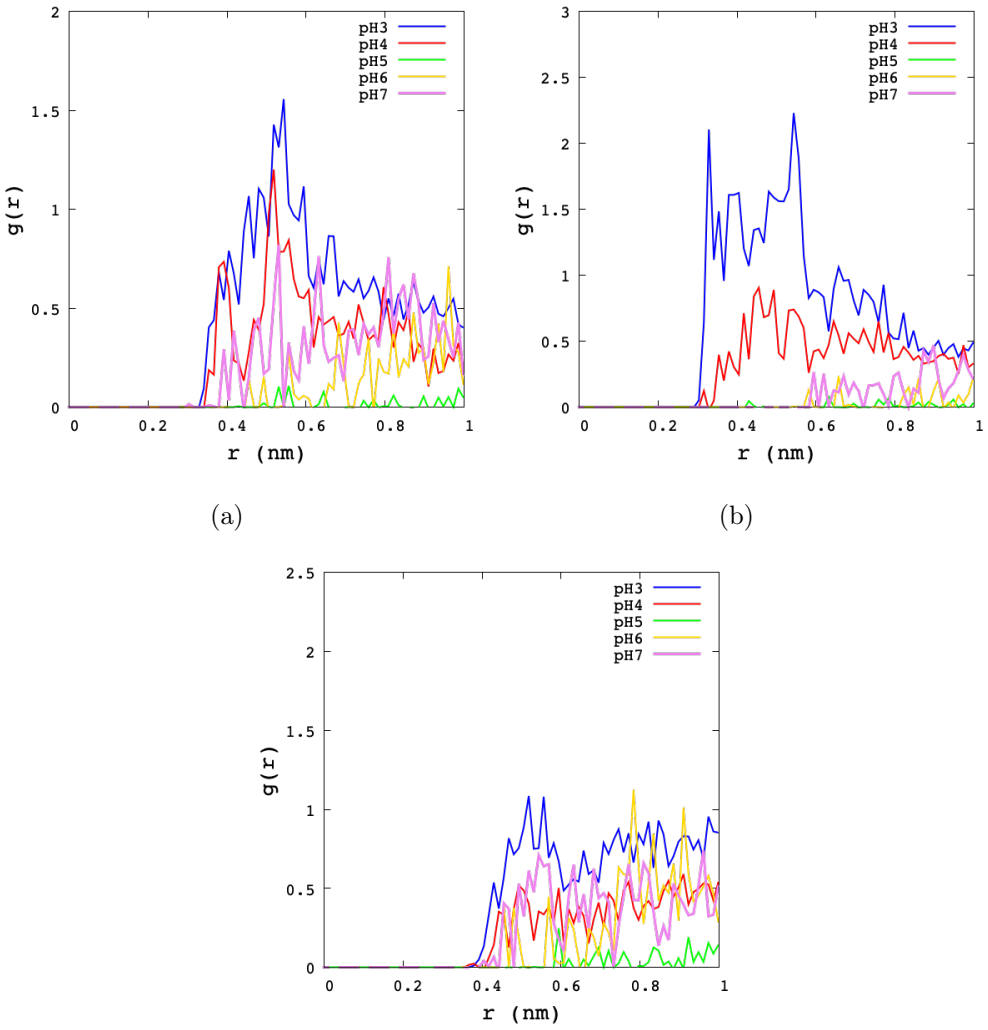

Figure S3. Radial Distribution of active site residues against Mpro. (a) Sγ–ions of Cys145.(b) Nδ1–ions of His41. (c) Sγ–ions of Met49.

Table S1. Total charge of each titratable amino acids.

pH3											
Residues	**MicroS-1**	**MicroS-2**	**MicroS-3**	**MicroS-4**	**MicroS-5**	**MicroS-6**	**MicroS-7**	**MicroS-8**	**MicroS-9**	**MicroS-10**	**Average value**
ASP (34)	-11	-9	-9	-8	-8	-8	-9	-7	-10	-8	**-(9±1)**
GLU (18)	-5	-5	-3	-4	-3	-3	-3	-6	-5	-5	**-(4±1)**
ARG (22)	22	22	22	22	22	22	22	22	22	22	**22**
LYS (22)	22	22	22	22	22	22	22	22	22	22	**22**
HIS (14)	10	10	9	10	10	10	10	10	10	10	**10**
pH4											
Residues	**MicroS-1**	**MicroS-2**	**MicroS-3**	**MicroS-4**	**MicroS-5**	**MicroS-6**	**MicroS-7**	**MicroS-8**	**MicroS-9**	**MicroS-10**	**Average**
ASP (34)	-21	-21	-27	-22	-22	-24	-20	-26	-22	-24	**-(23±2)**
GLU (18)	-6	-6	-6	-9	-8	-8	-6	-6	-7	-6	**-(7±1)**
ARG (22)	22	22	22	22	22	22	22	22	22	22	**22**
LYS (22)	22	22	22	22	22	22	22	22	22	22	**22**
HIS (14)	9	10	9	9	9	9	9	10	8	8	**9±1**
pH5											
Residues	**MicroS-1**	**MicroS-2**	**MicroS-3**	**MicroS-4**	**MicroS-5**	**MicroS-6**	**MicroS-7**	**MicroS-8**	**MicroS-9**	**MicroS-10**	**Average**
ASP (34)	-32	-30	-34	-33	-31	-33	-30	-34	-33	-32	**-(32±1)**
GLU (18)	-9	-13	-7	-9	-12	-11	-15	-10	-12	-10	**-(11±2)**
ARG (22)	22	22	22	22	22	22	22	22	22	22	**22**
LYS (22)	22	22	22	22	22	22	22	22	22	22	**22**
HIS (14)	6	5	7	5	3	7	4	7	5	7	**6±1**
pH6											
Residues	**MicroS-1**	**MicroS-2**	**MicroS-3**	**MicroS-4**	**MicroS-5**	**MicroS-6**	**MicroS-7**	**MicroS-8**	**MicroS-9**	**MicroS-10**	**Average**
ASP (34)	-34	-34	-34	-33	-33	-34	-34	-34	-34	-33	**-(34±1)**
GLU (18)	-16	-16	-14	-16	-15	-16	-17	-17	-15	-16	**-(16±1)**
ARG (22)	22	22	22	22	22	22	22	22	22	22	**22**
LYS (22)	22	22	22	22	22	22	22	22	22	22	**22**
HIS (14)	2	3	2	3	1	2	1	2	3	1	**2±1**
pH7											
Residues	**MicroS-1**	**MicroS-2**	**MicroS-3**	**MicroS-4**	**MicroS-5**	**MicroS-6**	**MicroS-7**	**MicroS-8**	**MicroS-9**	**MicroS-10**	**Average**
ASP (34)	-34	-34	-34	-34	-34	-34	-34	-34	-34	-34	**-34**
GLU (18)	-17	-17	-17	-16	-17	-17	-16	-17	-18	-18	**-(17±1)**
ARG (22)	22	22	22	22	22	22	22	22	22	22	**22**
LYS (22)	22	22	22	22	22	22	22	22	22	22	**22**
HIS (14)	1	0	0	0	1	2	0	0	1	1	**1±1**

Table S2. Protonate/deprotonate microstate of His41 and His172. The symbol P and D define the protonated and deprotonated states, respectively.

Residue	MicroS	pH3	pH4	pH5	pH6	pH7
His41	1	chainA(P)/chainB(P)	chainA(P)/chainB(D)	chainA(D)/chainB(D)	chainA(D)/chainB(D)	chainA(D)/chainB(D)
	2	chainA(P)/chainB(P)	chainA(P)/chainB(P)	chainA(D)/chainB(D)	chainA(D)/chainB(D)	chainA(D)/chainB(D)
	3	chainA(D)/chainB(P)	chainA(D)/chainB(P)	chainA(P)/chainB(P)	chainA(D)/chainB(D)	chainA(D)/chainB(D)
	4	chainA(P)/chainB(P)	chainA(P)/chainB(P)	chainA(D)/chainB(D)	chainA(D)/chainB(D)	chainA(D)/chainB(D)
His172	1	chainA(P)/chainB(P)	chainA(P)/chainB(P)	chainA(P)/chainB(P)	chainA(D)/chainB(D)	chainA(D)/chainB(D)
	2	chainA(P)/chainB(P)	chainA(P)/chainB(P)	chainA(D)/chainB(D)	chainA(D)/chainB(D)	chainA(D)/chainB(D)
	3	chainA(P)/chainB(P)	chainA(P)/chainB(P)	chainA(D)/chainB(D)	chainA(D)/chainB(D)	chainA(D)/chainB(D)
	4	chainA(P)/chainB(P)	chainA(P)/chainB(P)	chainA(D)/chainB(D)	chainA(D)/chainB(D)	chainA(D)/chainB(D)

Table S3. Values of the MD was fitted against the average structure of the last 100 ns of the dynamics was used.

pH	**MicroS**	**RMSD (nm)**	**RG (nm)**	**SASA (nm^2)**	**Hbond (protein-protein)**	**Hbond (protein-waters)**
pH3	pH3-1	0.28±0.02	2.543±0.009	269±3	404±9	(111±9)x10
	pH3-2	0.21±0.02	2.561±0.011	269±3	409±10	(103±5)x10
	pH3-3	0.32±0.05	2.543±0.019	266±5	393±10	(110±8)x10
	pH3-4	0.24±0.02	2.562±0.008	267±3	405±9	(109±9)x10
	Average	0.26±0.06	2.553±0.010	268±2	401±1	(107±4)x10
pH4	pH4-1	0.22±0.02	2.537±0.008	261±3	438±10	(109±10)x10
	pH4-2	0.21±0.02	2.538±0.008	262±3	427±9	(110±10)x10
	pH4-3	0.18±0.02	2.536±0.008	259±3	447±10	(109±9)x10
	pH4-4	0.21±0.02	2.541±0.009	263±4	432±10	(112±10)x10
	Average	0.20±0.02	2.538±0.002	261±2	437±10	(110±2)x10
pH5	pH5-1	0.25±0.03	2.532±0.009	258±3	448±9	(122±15)x10
	pH5-2	0.24±0.02	2.542±0.008	262±3	450±10	(109±9)x10
	pH5-3	0.24±0.02	2.518±0.008	256±3	445±10	(107±9)x10
	pH5-4	0.18±0.02	2.537±0.008	262±3	449±9	(115±11)x10
	Average	0.21±0.04	2.537±0.005	259±3	448±3	(114±8)x10
pH6	pH6-1	0.18±0.01	2.534±0.007	261±3	462±9	(102±2)x10
	pH6-2	0.22±0.01	2.527±0.007	258±3	450±9	(122±10)x10
	pH6-3	0.20±0.02	2.534±0.008	259±3	452±9	(126±12)x10
	pH6-4	0.21±0.02	2.537±0.008	259±3	458±8	(116±11)x10
	Average	0.20±0.02	2.532±0.005	260±2	456±6	(114±12)x10
pH7	pH7-1	0.16±0.02	2.533±0.010	259±3	460±10	(102±2)x10
	pH7-2	0.21±0.02	2.526±0.008	258±3	462±9	(126±10)x10
	pH7-3	0.21±0.03	2.528±0.008	260±3	460±9	(118±12)x10
	pH7-4	0.19±0.01	2.519±0.006	258±3	461±10	(121±12)x10
	Average	0.19±0.02	2.526±0.007	259±1	461±1	(114±12)x10

Table S4. Distances between amino acids from the catalytic dyad for each microstate.

pH	MicroS	His41-Cys145	Met49-Cys145	His172-Cys145
pH3	pH3-1	0.74±0.10	2.50±0.09	0.94±0.10
	pH3-2	0.71±0.11	1.59±0.18	0.93±0.16
	pH3-3	0.65±0.06	1.39±0.04	0.67±0.09
	pH3-4	0.50±0.03	1.37±0.05	0.85±0.04
	Average	0.65±0.04	1.71±0.05	0.85±0.05
pH4	pH4-1	0.61±0.08	1.62±0.14	1.20±0.04
	pH4-2	0.68±0.09	1.31±0.08	1.13±0.13
	pH4-3	0.57±0.07	1.53±0.19	1.21±0.06
	pH4-4	0.60±0.07	1.39±0.05	1.11±0.14
	Average	0.62±0.04	1.46±0.07	1.16±0.05
pH5	pH5-1	0.55±0.04	1.41±0.06	0.91±0.06
	pH5-2	0.59±0.06	1.41±0.06	0.72±0.08
	pH5-3	0.53±0.06	1.43±0.07	0.68±0.12
	pH5-4	0.54±0.07	1.43±0.06	0.87±0.12
	Average	0.55±0.03	1.42±0.03	0.79±0.05
pH6	pH6-1	0.54±0.08	1.43±0.06	1.06±0.07
	pH6-2	0.56±0.08	1.41±0.06	0.70±0.10
	pH6-3	0.54±0.06	1.41±0.07	0.89±0.10
	pH6-4	0.48±0.05	1.49±0.05	1.00±0.06
	Average	0.53±0.03	1.43±0.03	0.91±0.04
pH7	pH7-1	0.52±0.06	1.40±0.06	0.97±0.11
	pH7-2	0.50±0.06	1.46±0.07	0.93±0.08
	pH7-3	0.50±0.08	1.39±0.07	0.94±0.06
	pH7-4	0.54±0.03	1.38±0.06	0.81±0.05
	Average	0.51±0.03	1.41±0.03	0.91±0.04

Table S5. Numerical values of Ramachandran plot of the last frame of each microstate determined by Molprobity server.

	MicroS	Poor rotamers		Favored rotamers		Ramachandran outliers		Ramachandran favored		Favored	Allowed
pH3	pH3-1	9	1.80%	452	90.40%	6	0.99%	563	93.21%	93.20%	99.00%
	pH3-2	13	2.60%	456	91.20%	5	0.83%	564	93.38%	93.40%	99.20%
	pH3-3	13	2.60%	456	91.20%	7	1.16%	565	93.54%	93.50%	98.80%
	pH3-4	10	2.00%	452	90.40%	6	0.99%	567	93.87%	93.90%	99.00%
pH4	pH4-1	11	2.20%	455	91.00%	6	0.99%	565	93.54%	93.50%	99.00%
	pH4-2	10	2.00%	450	90.00%	5	0.83%	568	94.04%	94.00%	99.20%
	pH4-3	13	2.60%	445	89.00%	4	0.66%	558	92.38%	92.40%	99.30%
	pH4-4	10	2.00%	449	89.80%	4	0.66%	567	93.87%	93.90%	99.30%
pH5	pH5-1	15	3.00%	450	90.00%	2	0.33%	552	91.39%	91.40%	99.70%
	pH5-2	14	2.80%	449	89.80%	2	0.33%	562	93.05%	93.00%	99.70%
	pH5-3	14	2.80%	454	90.80%	7	1.16%	553	91.56%	91.60%	98.80%
	pH5-4	11	2.20%	459	91.80%	2	0.33%	555	91.89%	91.90%	99.70%
pH6	pH6-1	19	3.80%	439	87.80%	8	1.32%	556	92.05%	92.10%	98.70%
	pH6-2	13	2.60%	454	90.80%	6	0.99%	564	93.38%	93.40%	99.00%
	pH6-3	8	1.60%	451	90.20%	8	1.32%	554	91.72%	91.70%	98.70%
	pH6-4	12	2.40%	451	90.20%	5	0.83%	554	91.72%	91.70%	99.20%
pH7	pH7-1	13	2.60%	448	89.60%	3	0.50%	554	91.72%	91.70%	99.50%
	pH7-2	17	3.40%	457	91.40%	0	0.00%	562	93.05%	93.00%	100.00%
	pH7-3	10	2.00%	450	90.00%	5	0.83%	554	91.72%	91.70%	99.20%
	pH7-4	7	1.40%	466	93.20%	5	0.83%	556	92.05%	92.10%	99.20%

Table S6. Docking results of each microstate at different pHs.

Name	Size x (Å)	Size y (Å)	Size z (Å)	Search Domain Volume (A^3)	Number of flexible side chains
ph3-2-a	39.2	31.4	31.7	39018.896	32
ph3-2-b	40	33.2	32	42496	32
ph3-3-a	34.3	31.6	37.7	40862.276	32
ph3-3-b	38.4	33.8	27.8	36082.176	33
ph3-4-a	30.8	28.7	35.1	31026.996	32
ph3-4-b	31.6	33	31.9	33265.32	28
ph3-a	29.4	44	33.9	43853.04	27
ph3-b	42.7	37.9	33.6	54375.888	30
ph4-2-a	29.9	36.4	30.4	33086.144	28
ph4-2-b	31	29.5	29.8	27252.1	29
ph4-3-a	27.8	34.5	30.5	29252.55	27
ph4-3-b	28.9	30	33.3	28871.1	28
ph4-4-a	27.9	33.9	27.5	26009.775	29
ph4-4-b	29.8	29.2	37.7	32805.032	29
ph4-a	40	38	43	65360	31
ph4-b	37	40	48.1	71188	32
ph5-2-a	35	31.6	32	35392	30
ph5-2-b	32.1	36.2	35	40670.7	30
ph5-3-a	30.6	26.1	32.5	25956.45	31
ph5-3-b	28	32.2	37.8	34080.48	30
ph5-4-a	35.2	34.8	27.5	33686.4	30
ph5-4-b	25.8	32.5	31.8	26664.3	31
ph5-a	37.9	37.1	47.5	66789.275	30
ph5-b	37.2	37.8	37.8	53152.848	30
ph6-2-a	28.3	28.5	34.8	28067.94	30
ph6-2-b	30.6	32.2	30.5	30052.26	29
ph6-3-a	30.8	34.9	33.2	35687.344	28
ph6-3-b	36.8	30.9	30.7	34909.584	29
ph6-4-a	30.2	28.5	43	37010.1	30
ph6-4-b	30.2	31.5	32.4	30822.12	30
ph6-a	36	37.6	36.8	49812.48	30
ph6-b	34	32.1	46.6	50859.24	31
ph7-2-a	30	28.3	33.4	28356.6	30
ph7-2-b	33.9	37.7	28.8	36807.264	29
ph7-3-a	30	26.4	37.6	29779.2	30
ph7-3-b	35.3	28.1	26	25790.18	31
ph7-4-a	25.9	34.8	30.8	27760.656	31
ph7-4-b	32.8	29.3	32.5	31233.8	31
ph7-a	37	34.5	45.7	58336.05	30
ph7-b	45.2	41	35	64862	31

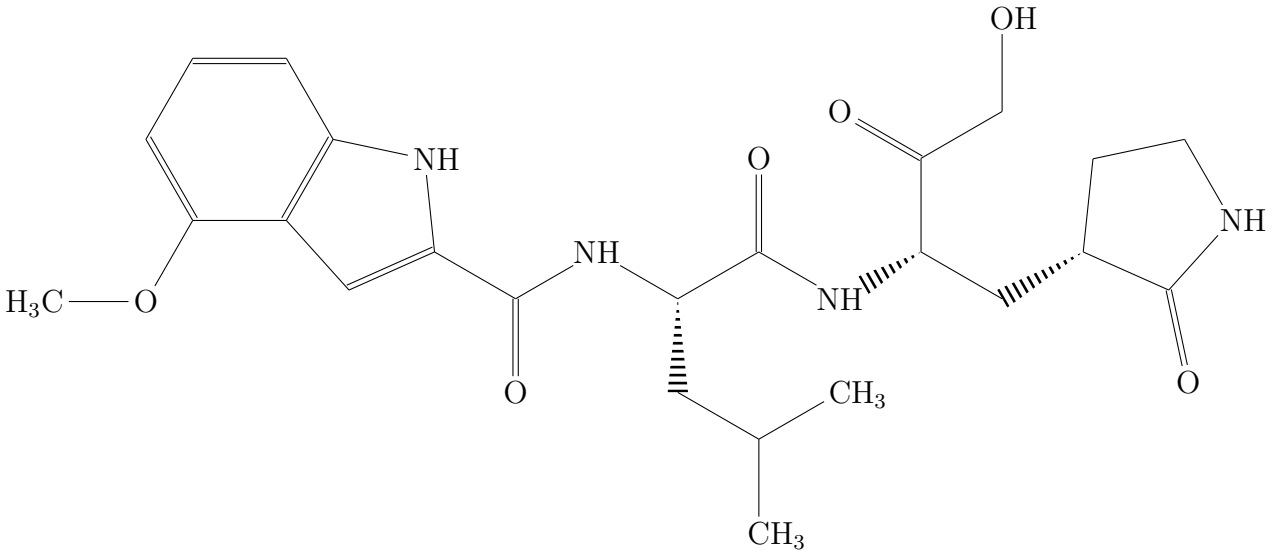

Figure S4. 2D representation of PF-00835231.

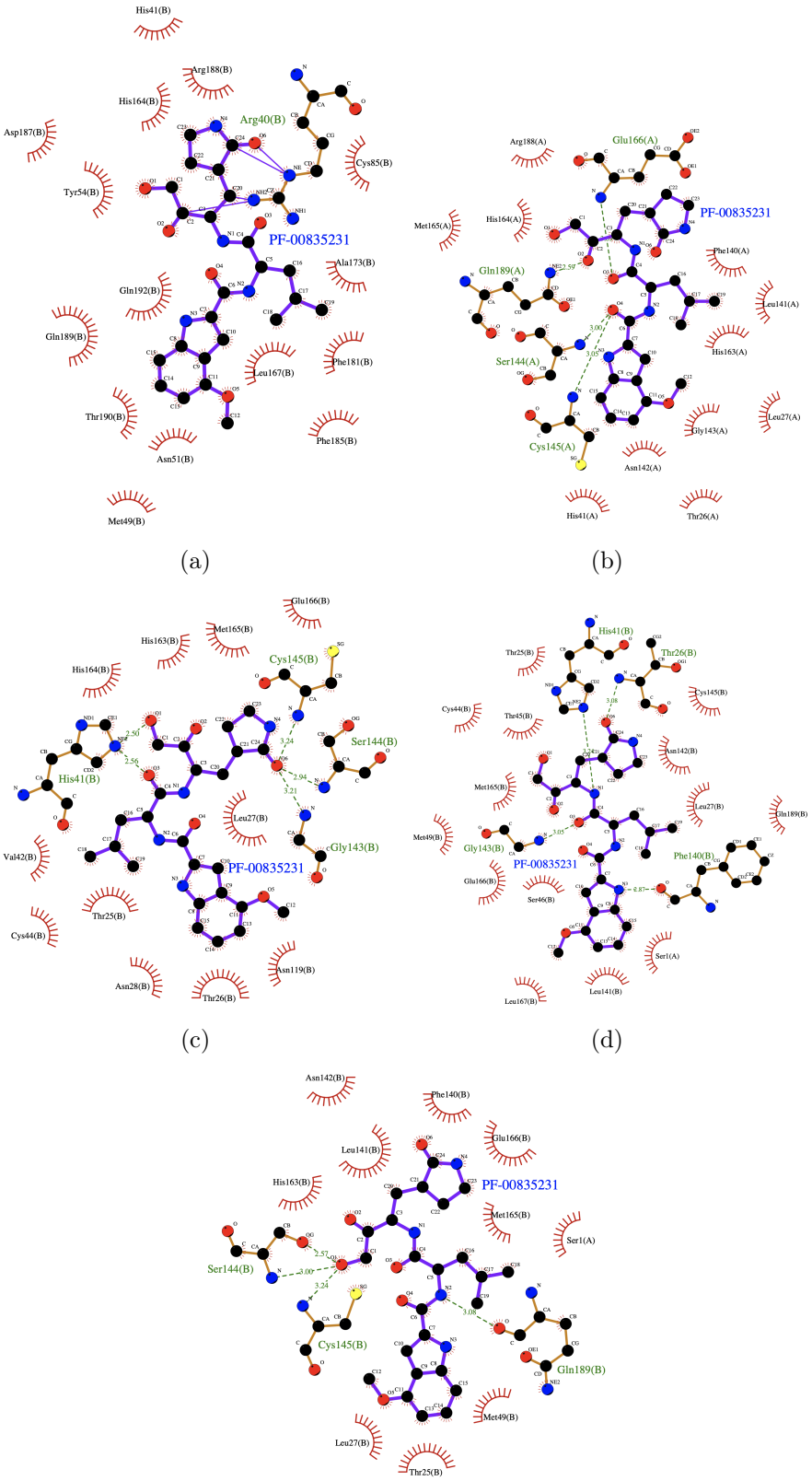

Figure S5. 2D representation of the best binding affinity Mpro–PF-00835231 . (a) pH3. (b) pH4. (c) pH5. (d) pH6. (e) pH7.