Supporting Information

Allosteric Modulation of HIV-1 Protease via Targeting the Flap-Tip Recognition Site

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Table S1. Anti-protease activity of Markush compounds

ID	Activity (%)	MW	ID	Activity (%)	MW
CB 5202063	80 ± 5	367	CB 9058855	68 ± 6	328
CB 5228968	--	306	CB 9112729	65 ± 7	324
CB 5343019	--	329	CB 9118639	72 ± 9	333
CB 5373492	65 ± 2	286	CB 9123846	79 ± 12	338
CB 5482954	--	352	CB 9154080	--	325
CB 5522020	88 ± 8	280	CCG 18156 [a]	52 ± 9	318
CB 5566364	76 ± 5	296	CCG 21102 [a]	42 ± 4	340
CB 5570164	--	339	CCG 24371 [a]	65 ± 6	400
CB 5660337	79 ± 2	310	CCG 29660 [a]	65 ± 8	439
CB 5660491	--	328	CCG 30993 [a]	62 ± 6	390
CB 5738653	84 ± 5	244	CD D071-0025	80 ± 7	343
CB 5797113	77 ± 7	279	CD D298-0425	82 ± 5	315
CB 5979646	37 ± 6	357	CD E245-0514	92 ± 5	336
CB 7386606	79 ± 9	342	CD E966-0066	--	358
CB 7910527	88 ± 4	317	CD E977-0196	88 ± 5	334
CB 7929697	71 ± 7	369	CD G428-0017	81 ± 11	354
CB 7932774	75 ± 4	325	CD G645-0045	91 ± 14	284
CB 7935081	64 ± 4	261	CD G851-1179	93 ± 6	314
CB 7936269	79 ± 10	256	MB BTB 09244	95 ± 8	297
CB 7936917	58 ± 5	282	MB HTS 12769	92 ± 8	274
CB 7937150	82 ± 7	328	MB HTS 12771	84 ± 7	280
CB 7940247	89 ± 5	299	MB HTS 12793	89 ± 12	347
CB 7989211	74 ± 6	304	MB SEW 04939	--	406
CB 9022153	78 ± 10	331			
CB 9058373	--	240			

CB: ChemBridge; CD: ChemDiv; IB: IBScreen; MB: MayBridge
All compounds were tested at 150 μM
[a] Compound not purchasable from chemical vendors

Table S2. Statistics of RMSD of protease in NIT-protease MD simulation trajectories						
	Run 1	Run 2	Run 3	Run 4	Run 5	Average
Median (Å)	1.99	1.68	1.86	1.98	2.16	1.93
St. Dev. (Å)	0.40	0.30	0.20	0.28	0.43	0.32
Maximum (Å)	3.52	2.85	2.65	2.72	4.05	3.16
Minimum (Å)	1.09	1.25	1.32	1.12	1.17	1.19

Table S3. Statistics of RMSD of NIT in NIT-protease MD simulation trajectories						
	Run 1	Run 2	Run 3	Run 4	Run 5	Average
Median (Å)	5.2	7.7	4.7	5.2	2.5	5.1
St. Dev. (Å)	2.4	1.3	1.4	2.4	0.7	1.6
	Box Plot Distribution of RMSD (Å)					
2.5 %	1.3	3.8	2.0	1.2	1.2	1.9
25 %	3.4	7.2	3.8	3.3	2.1	3.9
50 %	5.2	7.7	4.7	5.2	2.5	5.1
75 %	6.5	8.2	5.8	7.1	2.9	6.1
97.5 %	10.2	9.7	7.4	9.9	4.2	8.3

Table S4. Statistics of RMSD of protease in NIT-protease LD simulation trajectories						
	Run 1	Run 2	Run 3	Run 4	Run 5	Average
Median (Å)	2.84	2.53	2.54	3.27	2.44	2.72
St. Dev. (Å)	0.45	0.64	0.45	0.57	0.46	0.51
Maximum (Å)	4.07	5.13	4.04	4.74	4.06	4.41
Minimum (Å)	1.47	1.36	1.41	1.60	1.30	1.43

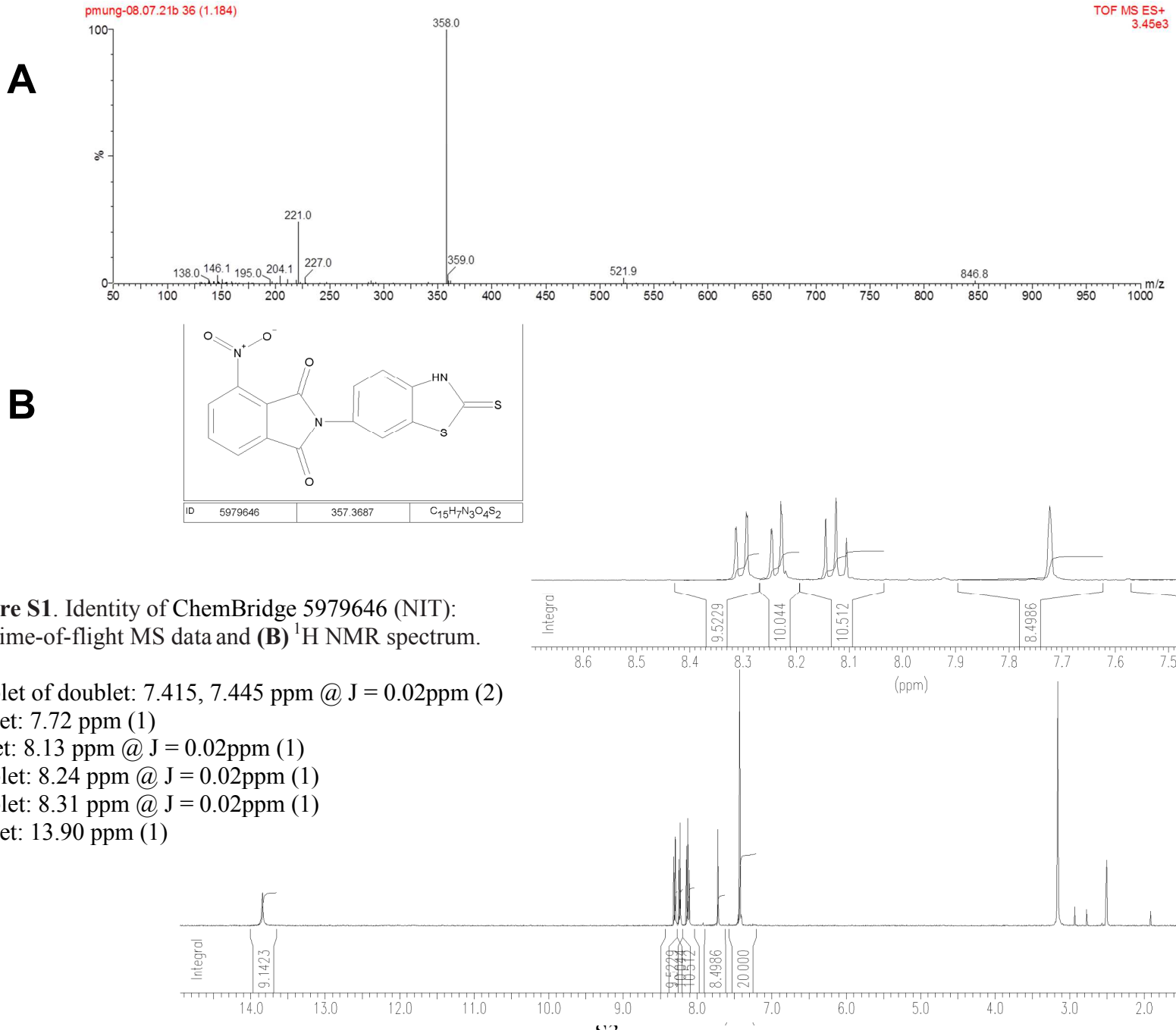

Figure S1. Identity of ChemBridge 5979646 (NIT): **(A)** time-of-flight MS data and **(B)** ^{1}H NMR spectrum.

doublet of doublet: 7.415, 7.445 ppm @ J = 0.02ppm (2)
singlet: 7.72 ppm (1)
triplet: 8.13 ppm @ J = 0.02ppm (1)
doublet: 8.24 ppm @ J = 0.02ppm (1)
doublet: 8.31 ppm @ J = 0.02ppm (1)
singlet: 13.90 ppm (1)

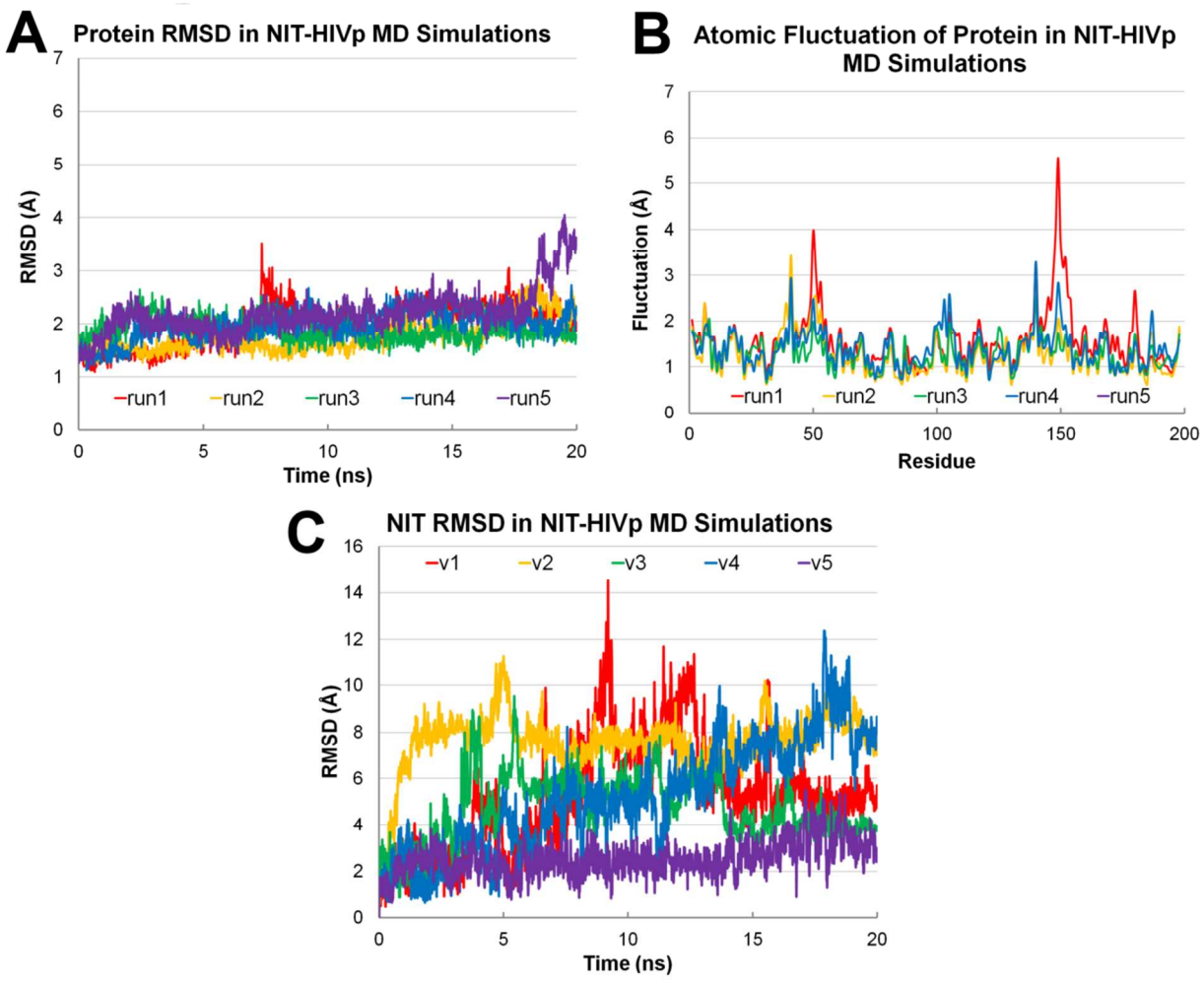

Figure S2. **(A)** RMS distance of the HIV-1p in five independent NIT-protease MD simulations throughout the production runs. The median RMSD of all five trajectories is 1.96 Å, with standard deviation of 0.32 Å and variance of 0.11 Å. **(B)** Atomic fluctuations by residue of NIT-protease MD simulations. The maximum fluctuations occur at the flap region (Ile47 – Phe53) on both monomers with the maximum fluctuation (> 2.0 Å) centered at residue Ile50. (**C**) RMS distance of the ligand NIT in the MD simulations.

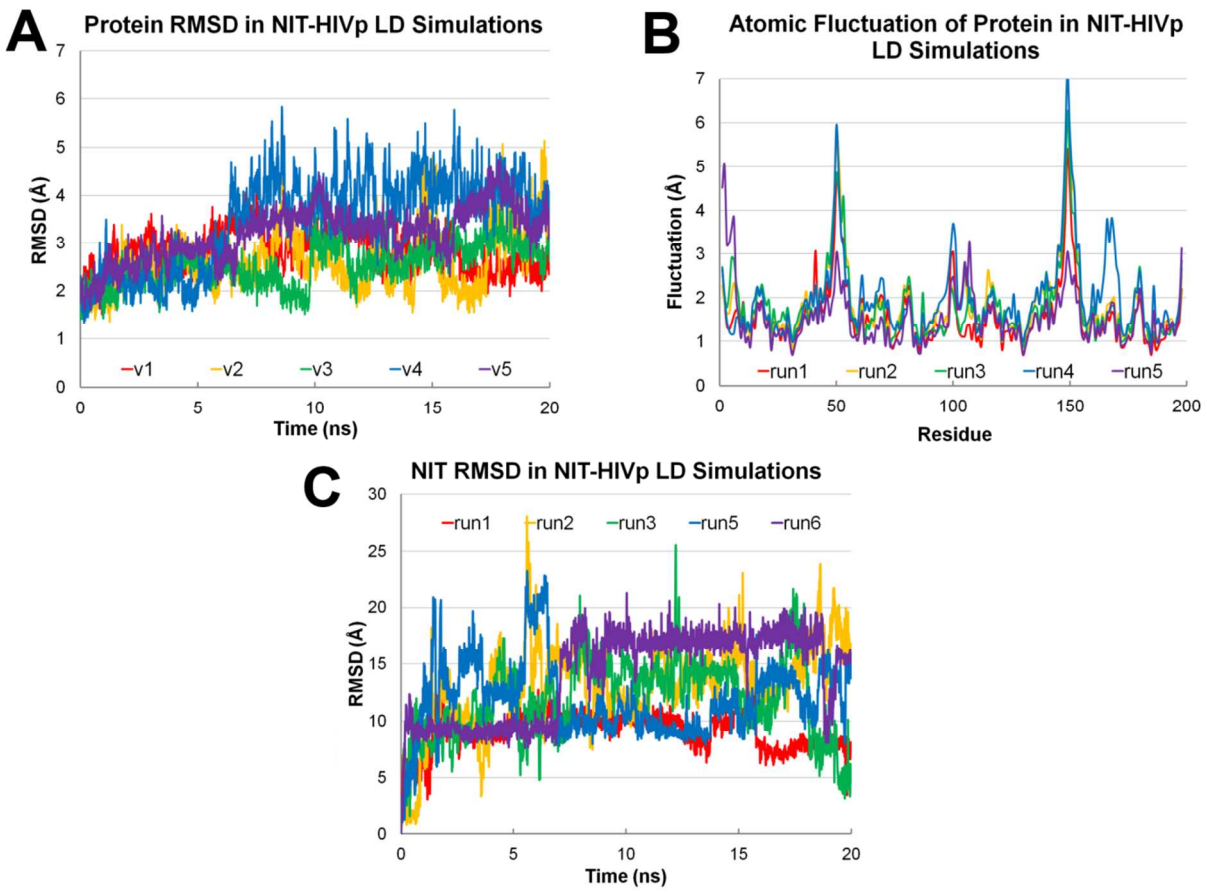

Figure S3. **(A)** Root-mean-square distance of five independent NIT-protein LD simulations throughout the production runs. The median RMSD of all five trajectories is 2.72 Å, with standard deviation of 0.51 Å and variance of 0.27 Å. **(B)** Atomic fluctuations by residue of five independent NIT-protease LD simulations. The maximum fluctuations occur at the flap region (Ile47 – Phe53) on both monomers with the maximum fluctuation (> 5.0 Å) centered at residue Ile50. (**C**) RMS distance of the ligand NIT in the LD simulations.