Supporting information

Structural Basis of the Allosteric Inhibitor Interaction on the HIV-1 Reverse Transcriptase RNase H domain.

Martin T. Christen[1+], Lakshmi Menon[1+], Nataliya S. Myshakina[2], Jinwoo Ahn[1], Michael A. Parniak[2], Rieko Ishima[1,*]
Department of Structural Biology[1] and Department of Microbiology and Molecular Genetics[2], University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

Supporting Information is available: Eight figures, including (1) the amino acid sequence of WT RNH used in this study, (2) Mg^{2+} effects on both monomer and dimer RNH, overlays of $^{1}H/^{15}N$ HSQC spectra of RNH monomer at different BHMP07 concentrations and the titration curves used for determination of the ligand dissociation constants in absence (3) and presence (4) of 20 mM Mg^{+}, histograms for (5) the ^{1}H and (6) the ^{15}N chemical shift perturbations induced by BHMP07 and Mg^{2+} on the RNH monomer, (7) diagram of the RNH residues predicted to surround BHMP07 in each of the three distinct docking sites, (8) per-residue interactions with BHMP07 as predicted for the minimum energy BHMP07 pose at Site II; and a table of individual K_D values for the interaction of RNH monomer with BHMP07.

```
1           11          21          31          41
|           |           |           |           |
SELYQLEKEP IIGAETFYVD GAANRETKLG KAGYVTDRGR QKVVPLTDTT
   |   |           |           |           |           |
  427 430         440         450         460         470


51          61          71          81          91
|           |           |           |           |
NQKTELQAIH LALQDSGLEV NIVTDSQYAL GIIQAQPDKS ESELVSQIIE
      |           |           |           |           |
      480         490         500         510         520


101         111         121         131
|           |           |           |
QLIKKEKVYL AWVPAHKGIG GNEQVDKLVS AGIRKVL
      |           |           |           |
      530         540         550         560
```

Figure S1. Explicit presentation of the amino acid sequence of the RT RNH fragment used in this study, *i.e.* residues 427–560, with an additional N-terminal peptide, S–E–L(underlined). Bold characters indicate the metal coordinating residues. The substrate handle region is highlighted (gray). Residues are numbered according to the construct used (top) and the entire RT protein (bottom).

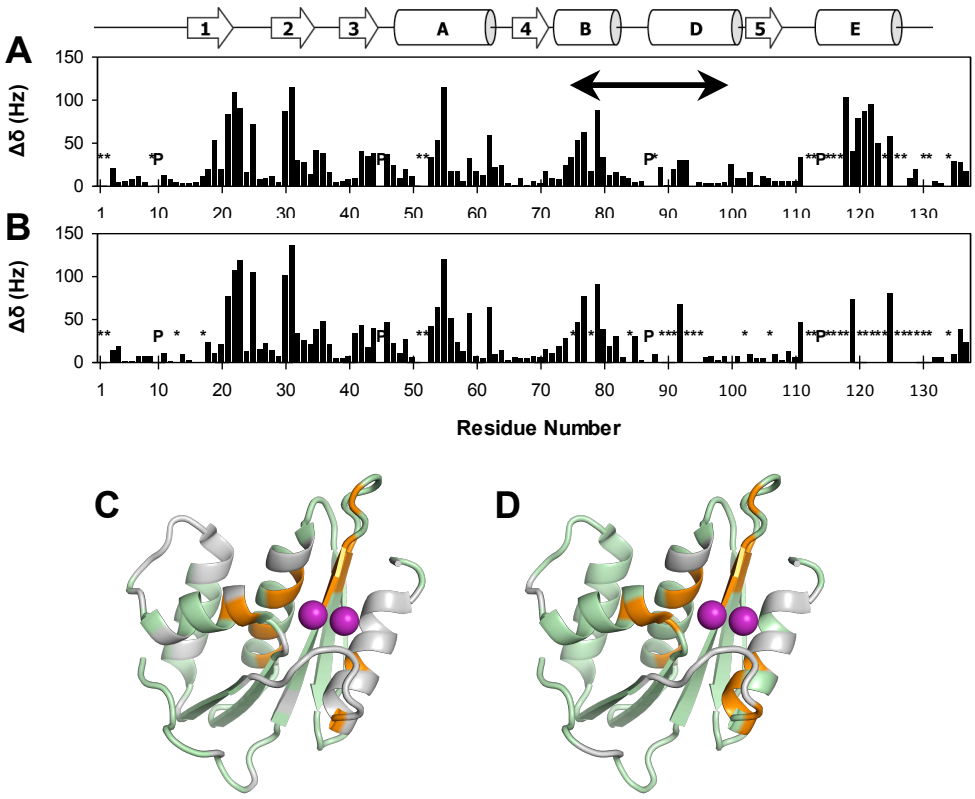

Figure S2. Chemical shift perturbations induced by Mg^{2+} on (A) monomer and (B) dimer RNH fractions, and their highlight on the ribbon structure presentations (C, and D, respectively). In (A) and (B), normalized quadratically weighed ^{1}H, ^{15}N backbone amide resonance shifts (Δδ, Hz) induced by addition of 20 mM $MgCl_2$ are shown relative to sequence residue; prolines (**P**) and unassigned/undetected NH amide groups (*) are indicated. In (C) and (D), residues exhibiting significant chemical shift changes ($\Delta\delta > \overline{\Delta\delta} + \sigma$) are highlighted in orange; undetected/unassigned residues are shaded in gray. These results provide compelling evidence that the dimerization process does not predominantly impede access to the active site.

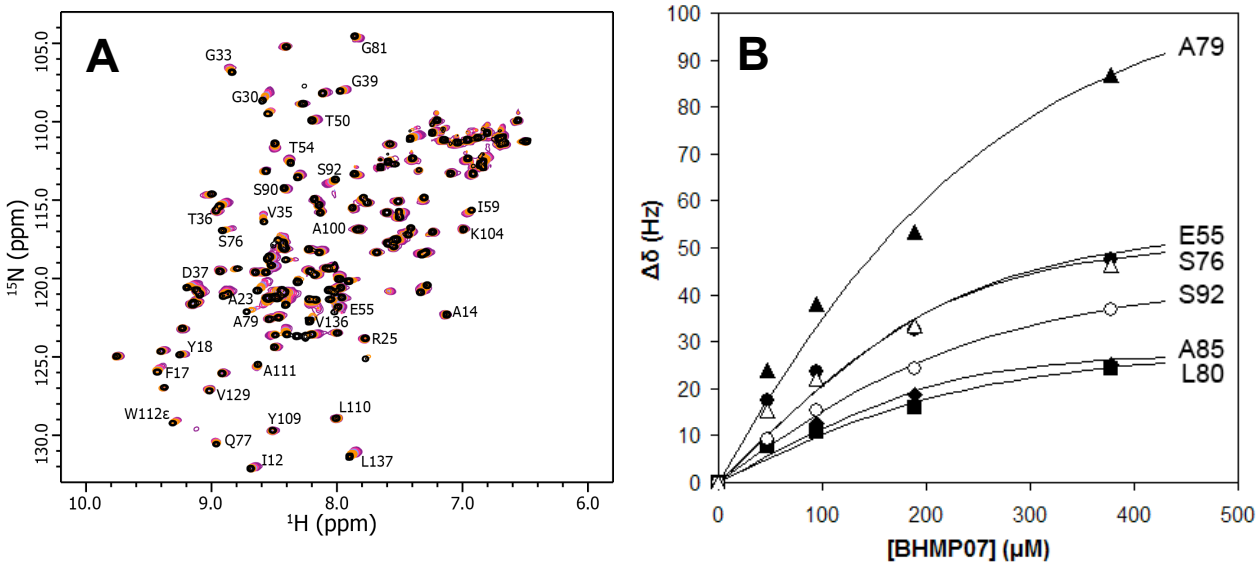

Figure S3. (A) Overlaid ^{1}H-^{15}N HSQC spectra of the RNH monomer recorded in absence (black) or presence of 0.2, 0.4, 0.8 and 1.6× BHMP07 (orange, blue, pink, and magenta, respectively). (B) Normalized chemical shift changes as a function of BHMP07 concentration for some representative residues, E55 (filled circle), A79 (filled triangle), L80 (filled squares), A85 (filled diamond), S92 (open circle), and S76 (open triangle). Data were recorded at 600 MHz (^{1}H), at 20°C and pH 7.0, protein concentration was ~ 230 μM.

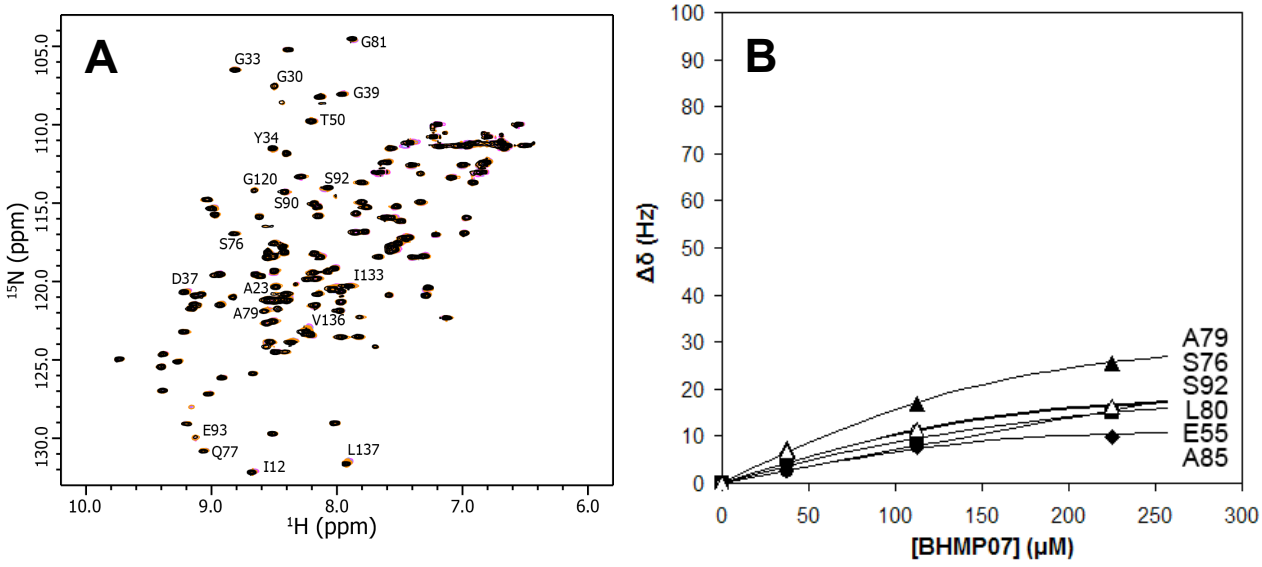

Figure S4. (A) Overlaid ^{1}H-^{15}N HSQC spectra of the RNH monomer after pre-addition of 20 mM $MgCl_2$ recorded in absence (black) or presence of 0.25, 0.5 and 1.5× BHMP07 (orange, light blue and magenta, respectively). (B) Normalized chemical shift changes as a function of BHMP07 concentration (same residues and representation as in **Figure S3B**). Data were recorded at 600 MHz (^{1}H), at 20°C and pH 7.0, protein concentration was ~ 150 μM.

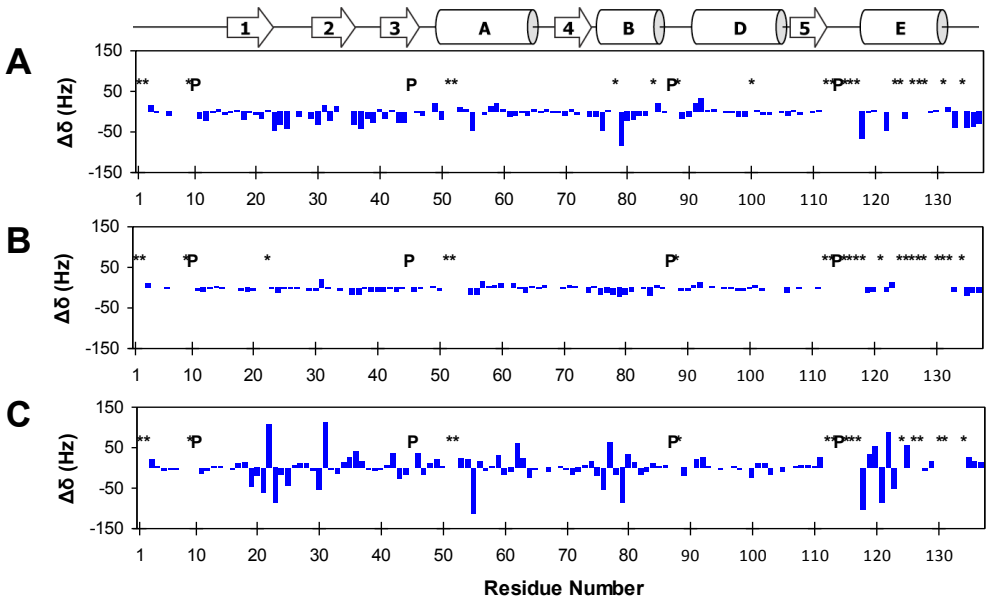

Figure S5. Histogram of backbone amide proton resonance shifts induced in RNH monomer by (A) BHMP07 in absence of Mg^{2+}, (B) BHMP07 in 20 mM $MgCl_2$ and (C) 20 mM $MgCl_2$ only. ^{1}H chemical shift changes (Δδ, Hz) are shown relative to sequence residue. Prolines (**P**) and unassigned/undetected NH amide groups (*) are indicated.

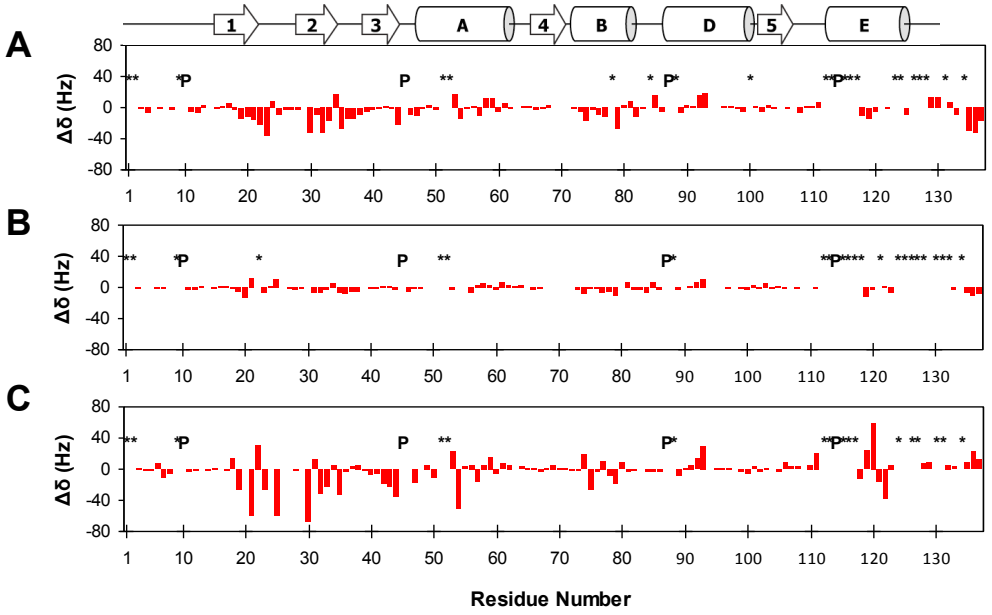

Figure S6. Histogram of backbone amide nitrogen resonance shifts induced in RNH monomer by (A) BHMP07 in absence of Mg^{2+}, (B) BHMP07 in 20 mM $MgCl_2$ and (C) 20 mM $MgCl_2$ only. ^{15}N chemical shift changes (Δδ, Hz) are shown relative to sequence residue. Prolines (**P**) and unassigned/undetected NH amide groups (*) are indicated.

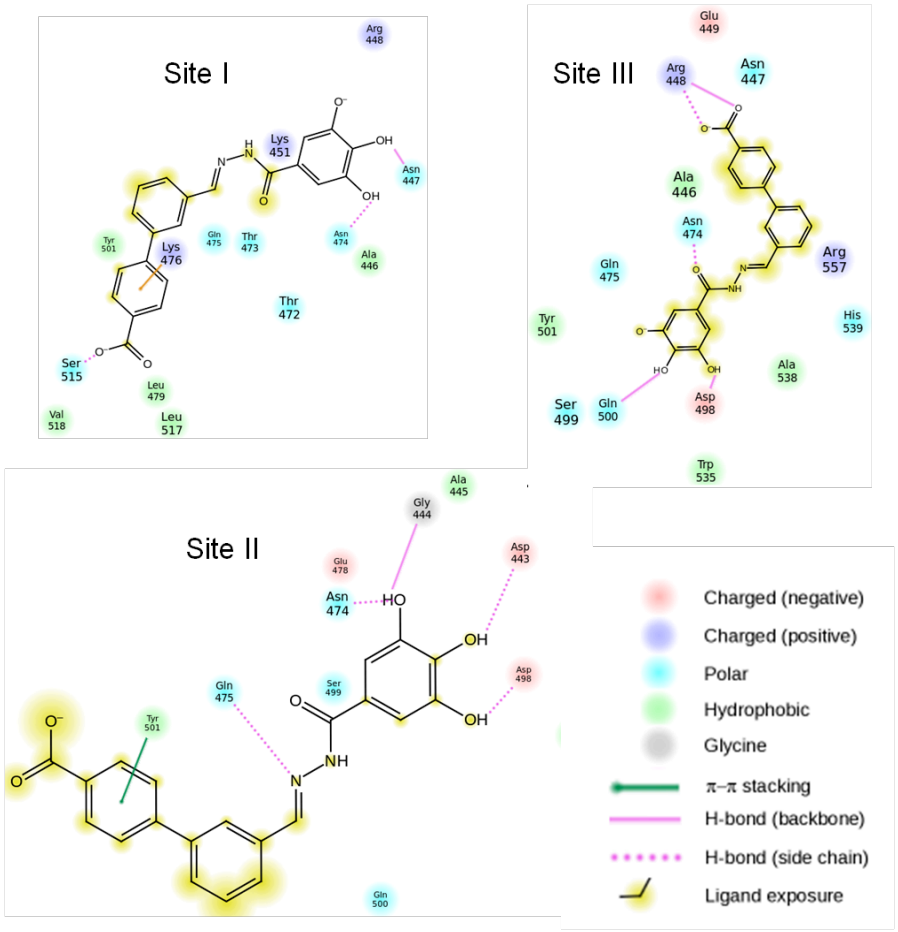

Figure S7. Ligand Interaction Diagrams for BHMP07 docked into RNH shown for each predicted binding site. Residues within 4.5Å proximity from the docked ligand are indicated and numbered according to the entire RT protein (see legend for coloring).

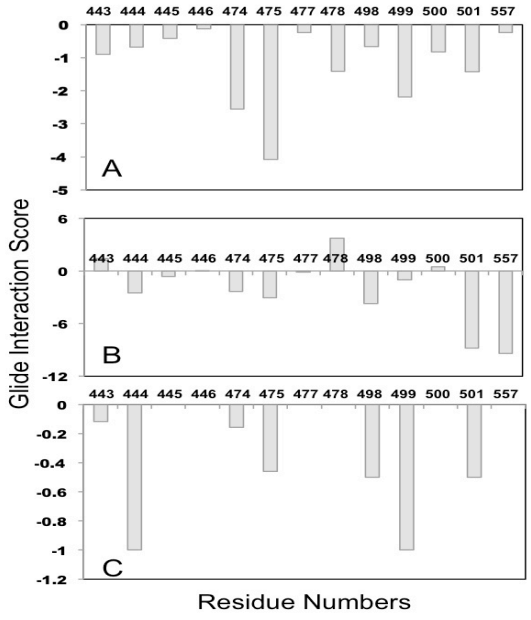

Figure S8. Per-residue interaction scores for BHMP07 bound to Site II in its best pose (highest docking score). (A) Van der Waals, (B) coulombic and (C) hydrogen bonding scores are shown: negative scores favor an interaction while positive scores represent penalties.

Table S1: Individual K_D for the Interaction of Monomeric RNH with BHMP07.

Residue [a]	K_D (mM) [b]	Δδ (Hz)	χ^2
3Leu	0.031 ± 0.048	17.4	7.7E-01
18Tyr	0.014 ± 0.017	20.6	1.2E+01
22Ala	0.008 ± 0.016	23.1	1.4E+01
23Ala	0.025 ± 0.005	58.1	3.0E+01
29Leu	0.106 ± 0.105	17.4	6.7E-01
30Gly	0.021 ± 0.004	46.8	2.5E+01
31Lys	0.067 ± 0.020	19.6	1.4E+01
32Ala	0.044 ± 0.010	39.8	1.4E+01
36Thr	0.023 ± 0.008	35.9	6.3E+00
37Asp	0.028 ± 0.007	45.2	1.1E+01
38Arg	0.038 ± 0.027	18.5	1.8E+00
39Gly	0.016 ± 0.012	26.4	1.0E+01
50Thr	0.187 ± 0.150	20.6	5.5E-01
55Glu	0.040 ± 0.007	47.5	1.8E+01
59Ile	0.009 ± 0.014	24.9	1.1E+01
74Thr	0.011 ± 0.015	21.4	1.4E+01
76Ser	0.029 ± 0.008	46.4	9.1E+00
79Ala	0.100 ± 0.017	86.9	2.2E+01
80Leu	0.061 ± 0.024	24.3	2.1E+00
81Gly	0.038 ± 0.025	21.4	1.9E+00
85Ala	0.021 ± 0.014	25.2	3.2E+00
92Ser	0.077 ± 0.033	36.9	1.1E+00
122Asn	0.011 ± 0.009	46.9	1.1E+01
133Ile	0.057 ± 0.016	42.2	5.0E+00
135Lys [c]	0.180 ± 0.025	48.0	1.3E+02
136Val	0.025 ± 0.005	49.7	3.8E+01

[a] Backbone amide NH resonances.

[b] The average K_D is 0.049 ± 0.025 mM.

[c] Peaks are broadened due to dynamic flexibility. Excluding this residue yields K_D = 0.043 ± 0.025 mM, *i.e.* within the error.