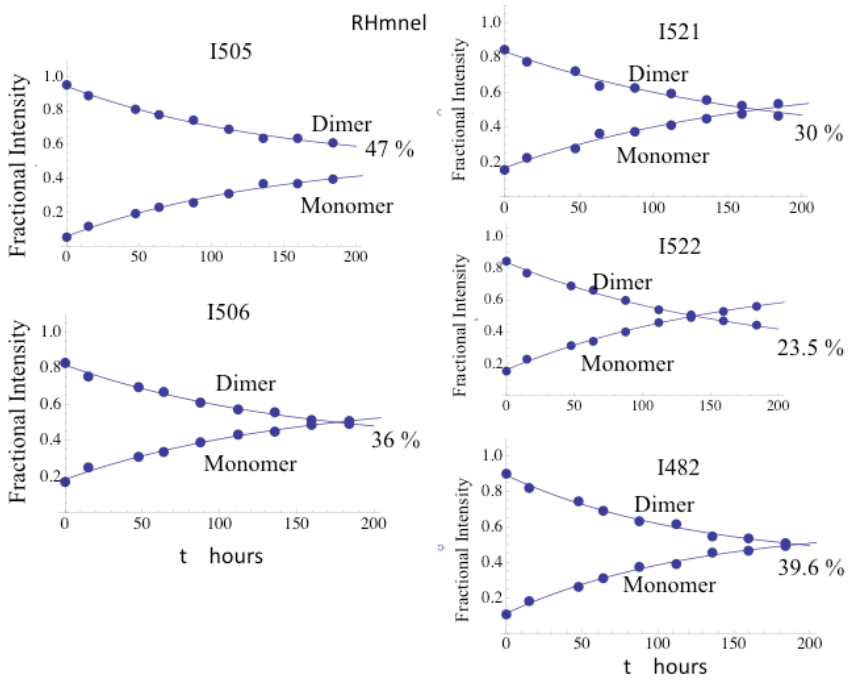
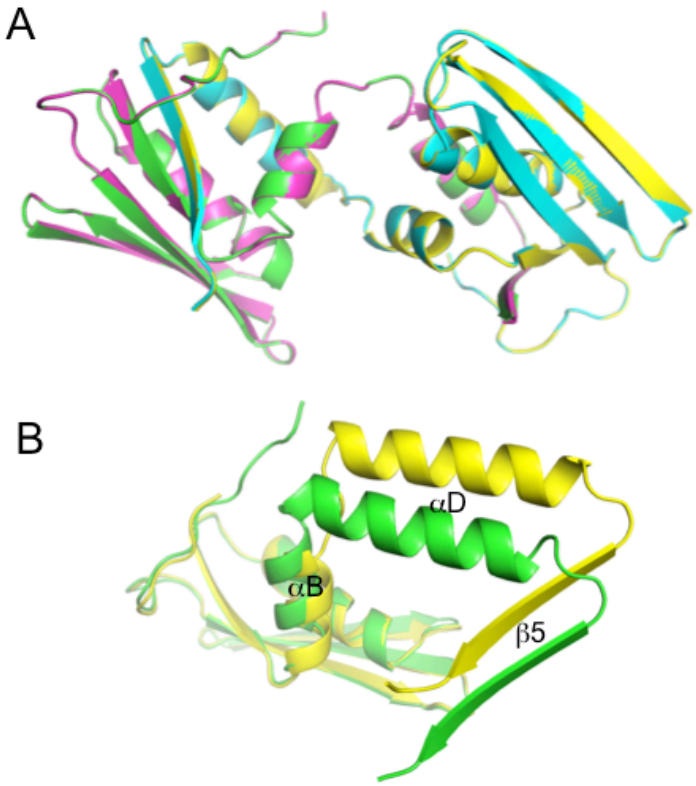
Supplementary Figures – Zheng et al., Unfolding the HIV-1 reverse transcriptase RNase H domain – how to lose a molecular tug-of-war

Supplementary Figure S1



Supplementary Figure S1. Time dependent behavior of normalized monomer and dimer intensities for Ile482, Ile505, Ile506, Ile521, and Ile522 δ-methyl resonances of RHmnel determined at 37 °C. The measurements correspond to a mean dimer decay time constant of 151.76 h, and a mean equilibrium dimer fraction of 0.35. Quantitative variations among the Ile resonance intensities may arise due to different relaxation characteristics between the monomer and dimer forms as well as some resonance overlap (see Figure 3).

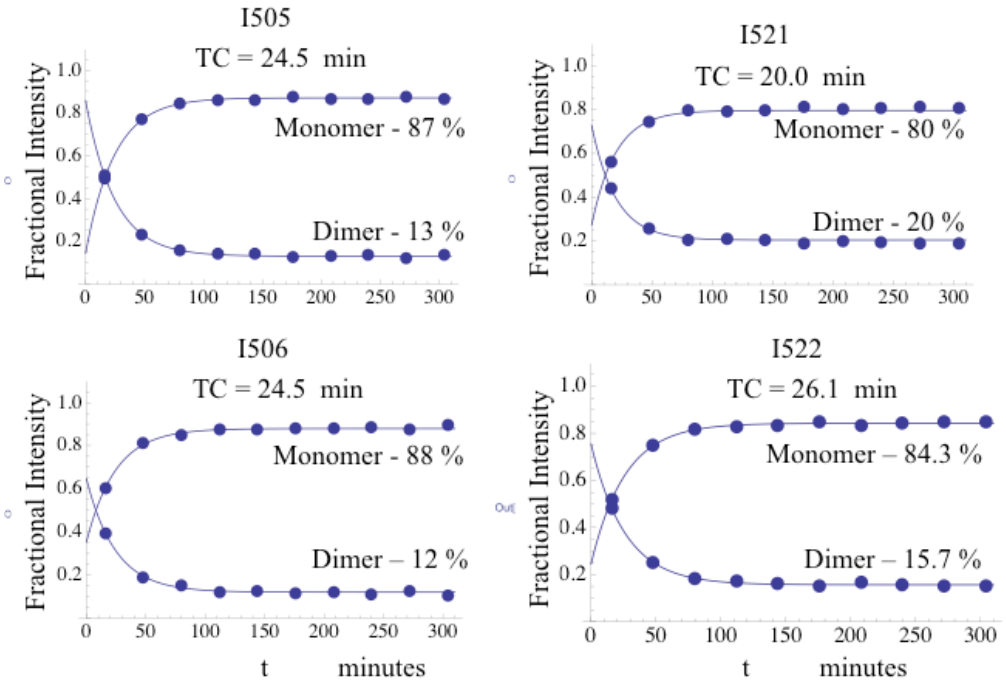
Supplementary Figure S2



Supplementary Figure 2. Structural comparisons with the RH dimer. A) Overlay of RH1-RH2 (green-yellow) with RH3-RH4 (magenta-cyan) showing equivalence of the domain swapped pair in the crystal. B) Overlaid ribbon diagrams of RH1 and RH2, aligned from residues 430-500. The B-D linker is somewhat longer in RH2 than in RH1, so that overlay of structural elements β1–β2–β3–αA–β4–αB results in a small displacement of the αD-β5 structural elements. Consequently, the two pseudo-monomers that are present in the dimer: β1–β2–β3–αA–β4–αB–αD'–β5' and β1'–β2'–β3'–αA'–β4'–αB'–αD–β5 differ primarily in position as well as in the orientation of αB. So to summarize, RH1-RH2 = RH3-RH4, however RH1 ≠ RH2 and RH3 ≠ RH4. However, these differences are substantially smaller than the differences relative to the RH monomer discussed in the text.

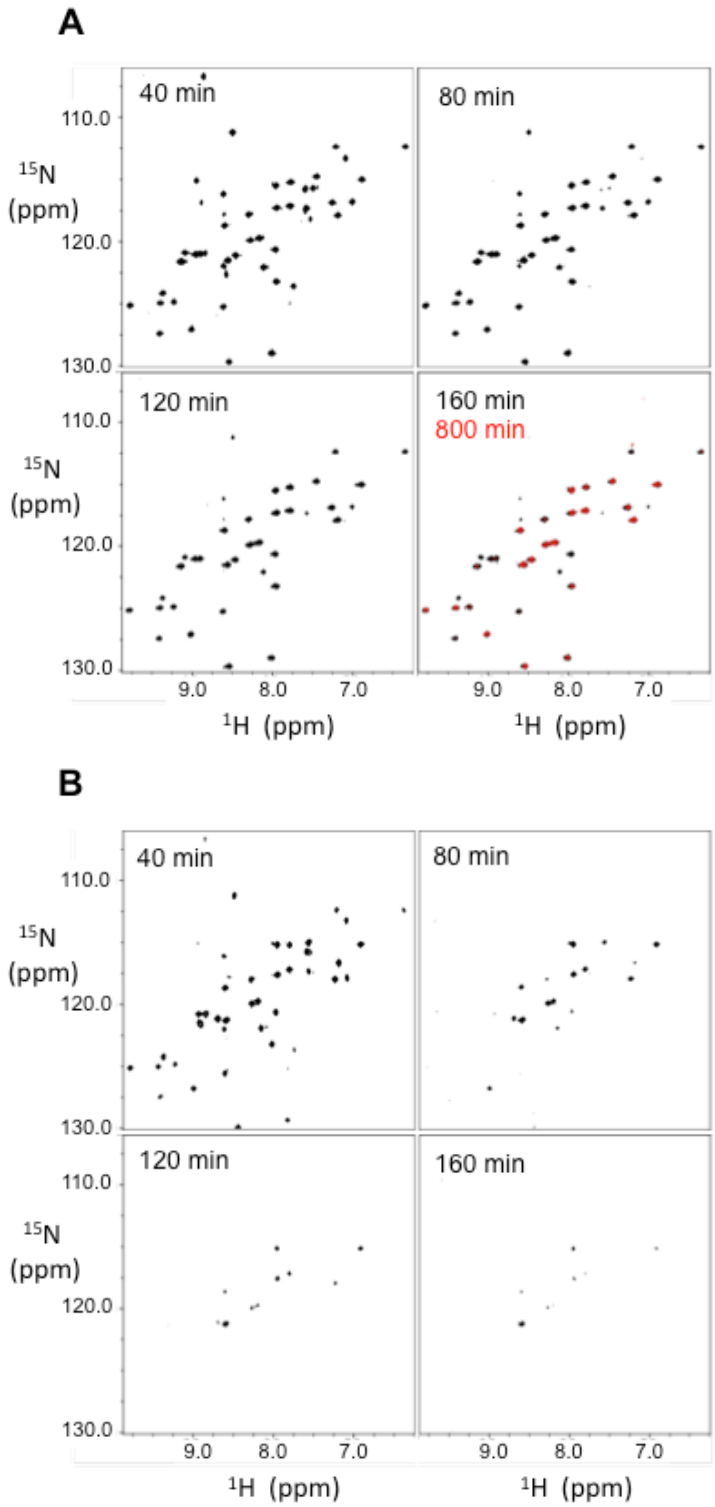
Crystal structure analysis indicates that the dihedral angles of the B-D loop residues 508-511 differ between the two monomers. TALOS+ analysis of the assigned dimer resonances reveal only one set of resonances, corresponding to dihedral angles that are in closer agreement with those observed for RH2 (chain B in pdb: 5DZM), but also indicate that some averaging may be occurring.

Supplementary Figure S3



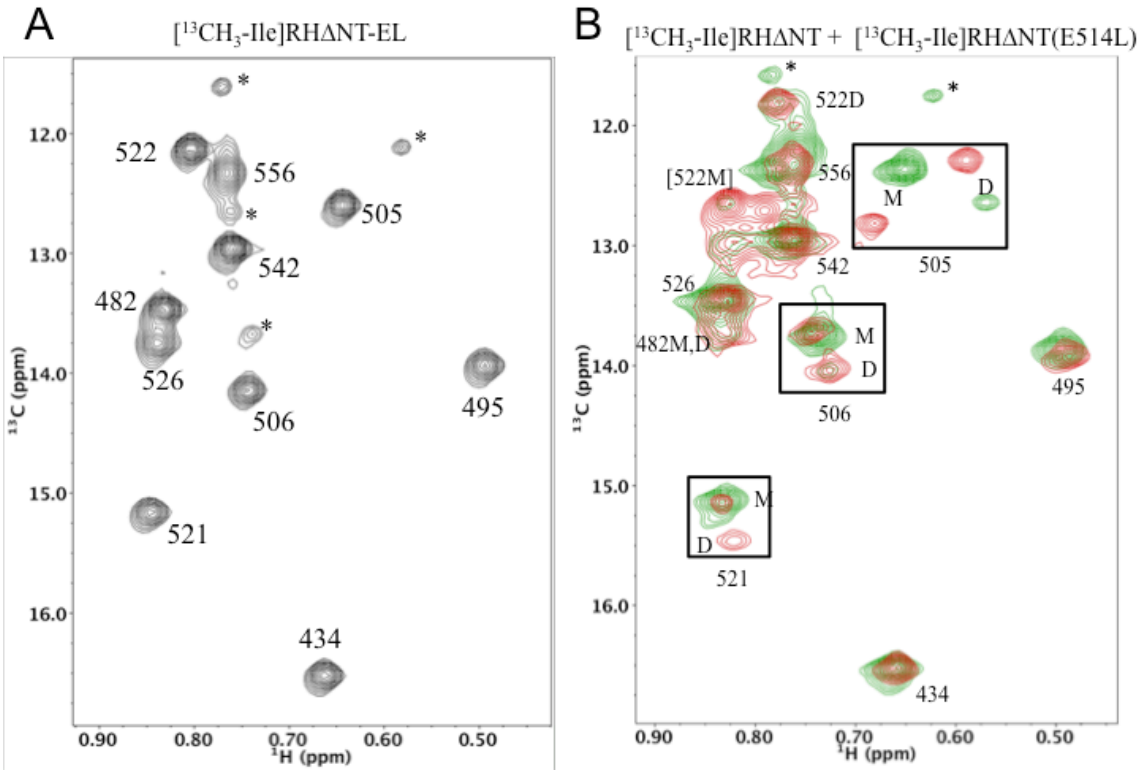
Supplementary Figure S3. Time dependent behavior of normalized monomer and dimer intensities for Ile505, Ile506, Ile521, and Ile522 δ-methyl resonances in RHΔNT. The rate constants are summarized in Table 1.

Supplementary Figure S4.



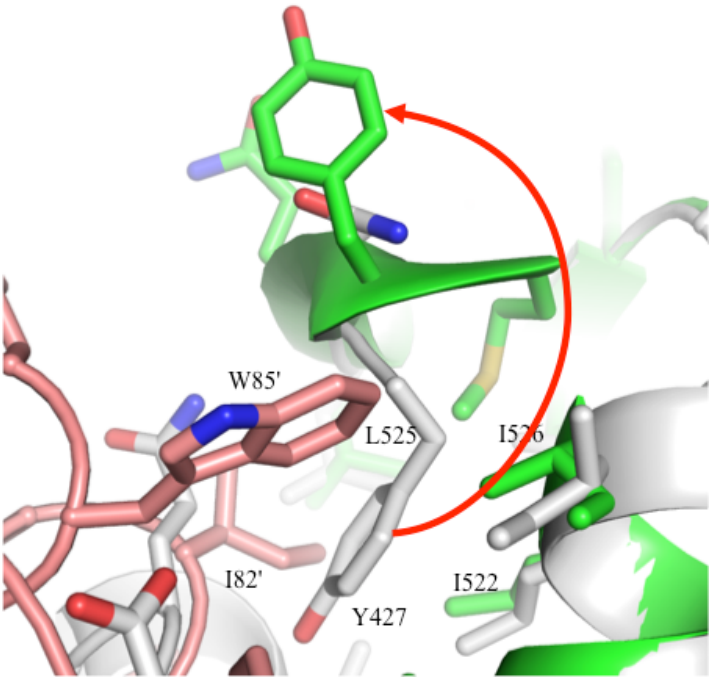
Supplementary Figure S4. ^{1}H-^{15}N HSQC spectra of RHmnel (A) and RHΔNT (B) obtained at the times indicated. Each spectrum was a 40 min acquisition. Samples were in 200 μM Tris-KCl-d11, pD 7.5, 100 mM KCl and run at 25 °C. As shown in the last spectrum of panel A, about 20 resonances remain with high intensity even at 800 min. In contrast, the spectra of RHΔNT obtained at 120 and 160 min indicate that most of the amide protons have been replaced by deuterons.

Supplementary Figure S5.



Supplementary Figure S5. Effect of linker mutations on the monomer/dimer ratio. A) ^{1}H-^{13}C HSQC spectrum of [^{13}CH$_3$-Ile]RHΔNT-EL containing an extended linker. Although the sample concentration was 450 μM and was allowed an overnight equilibration period, no dimer resonances were observed in the extended linker construct. B) Overlaid ^{1}H-^{13}C HSQC spectra of [^{13}CH$_3$-Ile]RHΔNT (green) and [^{13}CH$_3$-Ile]RHΔNT(E514L) (beige). Spectra were obtained after an equilibration period of 5 h. The assignment of the Ile522M resonance is tentative. The resolved resonances for Ile505, Ile506, and Ile521 each show that the dimer/monomer ratio is substantially greater in the E514L mutant, designed to favor transfer of residues from the B-D linker to helix αD.

Supplementary Figure S6.



Supplementary Figure S6. Variations in the Tyr427 binding pocket. The figure shows an overlay of ribbon diagrams for the RH domain monomer (pdb: 3K2P, gray), with the ribbon diagram representation of the HIV-E. coli chimera (pdb: 3HYF, green with fused E. coli basic protrusion in beige). In the chimeric structure, Tyr427 is displaced from its binding pocket due to steric conflicts with Ile82' and Trp85' from the E. coli insertion.