Supplementary Information

Ionization state of the catalytic dyad Asp25/25’ in the HIV-1 protease: NMR studies of site-specifically $^{13}$C labeled HIV-1 protease prepared by total chemical synthesis.

Vladimir Yu. Torbeev, Stephen B. H. Kent*

$^1$H-decoupled $^{13}$C-NMR spectra were acquired on Varian Unity Inova 900 spectrometer operating at 226.3 MHz for $^{13}$C-nucleus. For unliganded samples, typically ~5000 transients were collected with acquisition time 0.5 s and inter-pulse delay 1 s; for samples with inhibitors, 5000 – 10000 transients were acquired with acquisition time 0.5 s and inter-pulse delay varied from 2 s to 5 s (see legends of Fig. 2 and Supplementary Fig. 1 in the article). All samples were prepared in 18.9 mM Na.phosphate buffer (pH 5.7), containing 5.4 % (v/v) D$_2$O and 100 μM DSS-d$_6$. Inhibitors (MVT101 and KVS-1) were added in 4-fold molar excess to the solution of $[^L-Ala51/51']$HIV-1 protease after dialysis. Concentrations of protein were determined by integration of total LC-peak at 280 nm and were 0.34 mM for unliganded $[^L-Ala51/51']$HIV-1 protease, 0.41 mM for $[^D-Ala51/51']$HIV-1 protease, 0.29 mM for $[^Aib51/51']$HIV-1 protease, 0.4 mM for the complex of $[^L-Ala51/51']$HIV-1 protease with MVT-101 inhibitor, and 0.22 mM for the complex of $[^L-Ala51/51']$HIV-1 protease with KVS-1 inhibitor. $^{13}$C chemical shifts were referenced indirectly to DSS-d$_6$ using $\gamma_C/\gamma_H$ ratio.$^1$ For all experiments the temperature was set to 3.3 °C to slow down autoproteolysis. (1,4)-$^{13}$C-Aspartic acid was purchased from Cambridge Isotopes, and was then side-chain-protected with allyl group$^3$ and Boc-protected at the alpha amino group, and incorporated at Asp25 into the appropriate synthetic peptide segment as described.$^2$ Site-specific $^{13}$C-labelling was performed by total chemical protein synthesis, as previously described.$^2$ See Supplementary Figure 2 and Supplementary Figure 3 for LC-MS analytical characterization of the chemically synthesized full-length [1-99] HIV-1 protease wild-type and analogue polypeptide chains.
**Supplementary Figure 1.** 226 MHz $^{13}$C-$^1$H NMR spectra for complex of $[	ext{L-Ala51/51}'$H]HIV-1 protease with the reduced isostere inhibitor MVT-101, acquired with inter-pulse delay of 5 s (in a) and 2 s (in b). Red asterisk indicates peaks originating from unfolded peptidic autoproteolysis products. In (b) spectrum is dominated by autoproteolytic product and signals originating from protein are relaxation-filtered by application of the shorter inter-pulse delay.
Supplementary Figure S2. Analytical HPLC (λ = 214 nm) and ESI mass-spectra of $^{13}$C-labeled (a) wild-type [1-99]HIV-1 protease, and (b) L-Ala51 [1-99]HIV-1 protease.
Supplementary Figure 3. Analytical HPLC (λ = 214 nm) and ESI mass-spectra of $^{13}$C-labeled (a) $D$-Ala51 [1-99]HIV-1 protease, and (b) Aib51 [1-99]HIV-1 protease.
References: