Supporting Information:

Mutations Proximal to Sites of Autoproteolysis and the $\alpha$-Helix that Co-evolve under Drug Pressure Modulate the Autoprocessing and Vitality of HIV-1 Protease

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Figure S1. Evaluation of the autodigestion, $K_{dimer}$ and thermal melting of mature proteases. Published results of the time course of autoproteolysis of PR (A) and PR20 (B) by SDS-PAGE are shown here solely for comparison with PR^{PR20-123} and PR20^{PR-123} described in the main text.\(^1\) $K_{dimer}$ of PR^{PR20-123} (B) and PR20^{PR-123} (C) in 50 mM sodium acetate, pH 5.0, containing 250 mM sodium chloride at 28 °C. $K_{dimer}$ values were determined by fitting a previously described equation\(^2\) (shown below) to rate data, where PR\textsubscript{mono} is the concentration of PR expressed as monomers, $V_x$ is the observed initial rate/[PR\textsubscript{mono}], and $V_{max}$ is the extrapolated maximum rate/[PR\textsubscript{mono}] when the enzyme is fully dimeric. Data shown are scaled relative to a maximum activity of 100%.

$$V_x = V_{max} \left[ 1 - \left\{ \frac{(K_{dimer}/2 * [PR_{mono}])^{1/2} * ((K_{dimer}/2 * [PR_{mono}]) + 4)^{1/2} - (K_{dimer}/2 * [PR_{mono}])^{1/2}}{2} \right\} \right]$$

DSC thermograms of PR^{PR20-123} (E) PR20^{PR-123} (F) and their parent constructs PR and PR20, in the presence (solid lines) and absence (dashed lines) of a two-fold molar excess of darunavir. Data from previous work are shown for PR\(^3\) and PR20\(^1\) for comparison only.
Figure S2. Graphical representation of table 1.
Figure S3

**Figure S3.** Michaelis-Menten plots for hydrolysis of chromogenic substrate (measured at 310 nm) by 0.3 µM PR\textsuperscript{PR20-123} (A) and PR\textsuperscript{PR-123} (B) in 50 mM sodium acetate, pH 5.0, containing 250 mM sodium chloride at 28 °C. As higher substrate concentrations (> 450 µM) lead to weak inhibition by one of the cleavage products, the highest substrate concentration that could be used is below $K_m$ for PR\textsuperscript{PR20-123} (A). Thus, only the estimated lower limits for these kinetic parameters are given. For PR\textsuperscript{PR-123} (B) both Michaelis-Menten and Lineweaver-Burk plots are shown.
Figure S4. Determination of $K_i$ ($1/K_{\text{association}}$) for binding of inhibitors to PR$_{PR20-123}$ and PR$_{PR20-123}$ by ITC in 50 mM sodium acetate, pH 5.0, at 28 °C. For competitive inhibitors, $1/K_{\text{association}}$ for inhibitor binding by ITC is the same as determined kinetically. The apparent stoichiometry (N-value, indicated by the midpoint of the binding isotherm) for both titrations of PR$_{PR20-123}$, was lower than expected for the 1:1 ratio of the enzyme-inhibitor complex, likely due to autoproteolysis (see Figure 2). Therefore, the concentration was scaled in panels (C) and (D) to give an N-value of 1. No concentration correction was applied for PR$_{PR20-123}$ [panels (A) and (B)] as expected. DRV, SQV and RPB denote darunavir, saquinavir and reduced peptide bond inhibitor, respectively.
**Figure S5.** $K_i$ determination for reduced peptide bond inhibitor (RPB) binding to (A) 0.5 µM PR20 and (B) 0.6 µM PR$^{PR20-123}$ by use of Dixon plots for hydrolysis of chromogenic substrate. Each complete data set comprising 2 or 3 substrate concentrations was processed together by use of the enzyme kinetics module of Sigmaplot 10. (C and D) Kinetic determinations of IC$^{50}$ and $K_i$ for saquinavir (SQV) mediated inhibition of 0.54 µM PR20$^{PR-123}$ at two substrate concentrations. For duplicate measurements average values with error bars are shown. The solid lines are curve fits of the Morrison equation$^5$ (shown below) to the data with parameters IC$^{50}$ and $E$, where $V_o$ and $V_{obs}$ are initial rates in the absence and presence of inhibitor, respectively, and I and E are total concentrations of inhibitor and active sites respectively.

$$\frac{V_{obs}}{V_o} = 1 - \left\{ \left[ I + E + IC^{50} - \left[ (I + E + IC^{50})^2 - 4*I*E \right]^{1/2} \right]/2*I \right\}$$

$K_i$ values shown were calculated from IC$^{50}$ by use of the equation $K_i = IC^{50}/(1 + [Substrate]/K_m)$. 
Figure S6

Figure S6. 600 MHz $^1$H-$^{15}$N TROSY correlation spectra of freshly prepared $^{15}$N or $^{15}$N/$^{13}$C labeled proteins in 20 mM sodium phosphate buffer, pH 5.7, 20 °C. Spectra were acquired in the presence of the symmetric inhibitor DMP323. After acquiring the NMR spectra, samples were subjected to SDS-PAGE on homogeneous 20% Phastgels and stained with Phastgel blue R to visualize the bands. Inset lanes B and C denote PR20$^{PR-12}$ + DMP323 and PR20$^{PR-12}$, respectively. PR20$^{PR-12}$ undergoes rapid autoproteolysis in the absence of DMP323. M, FL and p denote molecular weight standards in kDa, full-length mature protease and products of autoproteolysis, respectively.
REFERENCES


