Supplementary Material

The Folding Free Energy Surface of HIV-1 Protease: Insights into the Thermodynamic Basis for Resistance to Inhibitors

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Table S1. Thermodynamic parameters for the urea-induced equilibrium folding reactions of HIV-PR* and mHIV-PR* at pH 6.0 and 25 °C.

	$\Delta G°(H_2O)$[a]	*m* value[b]
HIV-PR*[c]	-14.23 ± 0.23	2.89 ± 0.08
mHIV-PR*[d]	-1.35 ± 0.05	1.45 ± 0.24

[a]Units of kcal (mol dimer)$^{-1}$ for HIV-PR* and kcal (mol monomer)$^{-1}$ for mHIV-PR*.

[b]Units of kcal (mol dimer)$^{-1}$ M^{-1} for HIV-PR* and kcal (mol monomer)$^{-1}$ M^{-1} for mHIV-PR*.

[c]Equilibrium stability and *m* value obtained from globally fitting a set of 20 SVD vectors from equilibrium titrations monitored by CD and FL to the model $2U \leftrightarrows N_2$.

[d]Equilibrium stability and *m* value of the monomer construct, mHIV-PR*, obtained from a two-state fit of CD data to the model $U \leftrightarrows M$.

Supplementary Figure Legends

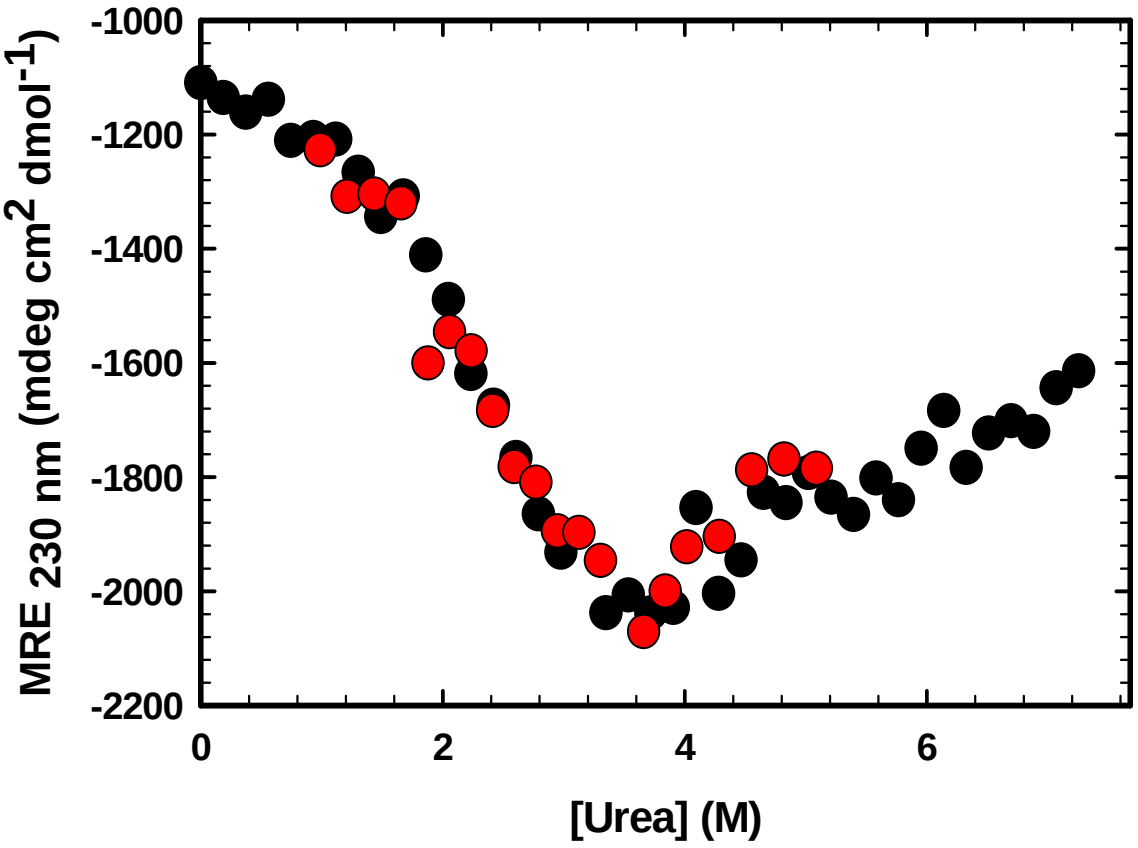
Figure S1. Reversibility of HIV-PR* folding. The equilibrium folding transition monitored by CD at 230 nm is shown as a function of urea concentration. Native (black circles) and unfolded (red circles) HIV-PR* were equilibrated in varying amounts of urea for 12 hours before measurement. Protein concentration in monomer units was 5 μM, and the buffer conditions were 100 mM sodium phosphate, pH 6.0, and 0.2 mM EDTA at 25 °C.

Figure S2. HIV-PR* folding and unfolding kinetics. (a) Unfolding and (b) refolding CD kinetic traces collected at 230 nm and several final urea concentrations. Lines through the data represent fits to one or two exponentials. Arrows indicate the initial MRE's of the native and unfolded species in unfolding (panel a) and refolding (panel b) jumps, respectively. (c) Unfolding and (d) refolding FL kinetic traces collected at 350 nm and several final urea concentrations. Lines through the data represent fits to three exponentials. Unfolding jumps were initiated in 0 M urea, and refolding jumps were initiated in 5 M urea. The final protein concentration was 4 μM. Buffer conditions are described in the caption to Figure S1.

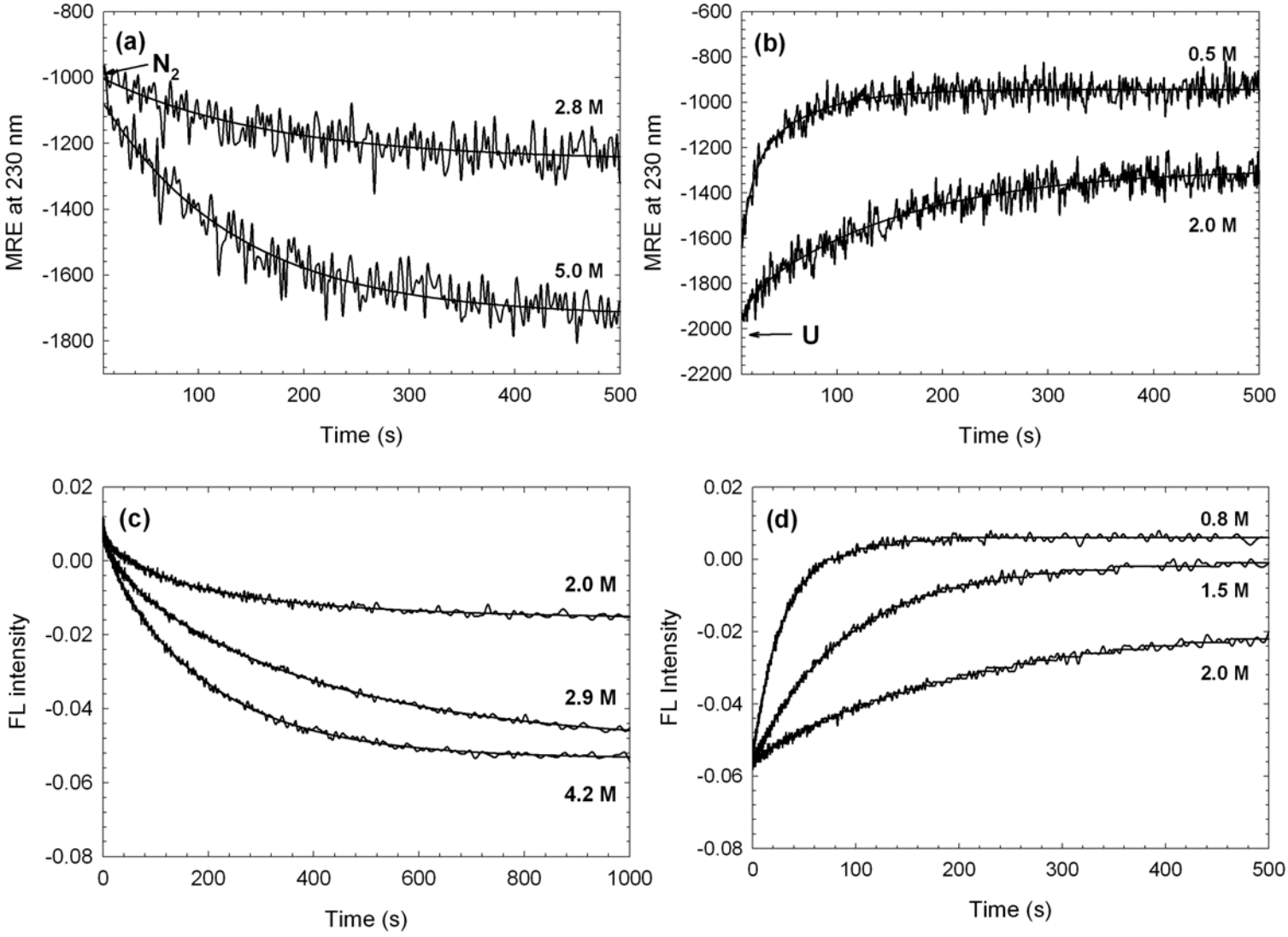
Figure S3. Protein concentration dependence of refolding relaxation times for HIV-PR*, monitored by stopped-flow fluorescence. Relaxation times, τ_s (filled circles), τ_i (filled squares), and τ_f (filled triangles), for refolding to 1 M urea are shown as a function of protein concentration. Of the three observed refolding phases, only τ_i exhibited a significant protein

concentration dependence. τ_i becomes the rate-limiting phase at nanomolar protein concentrations. The buffer conditions are described in the caption to S1.

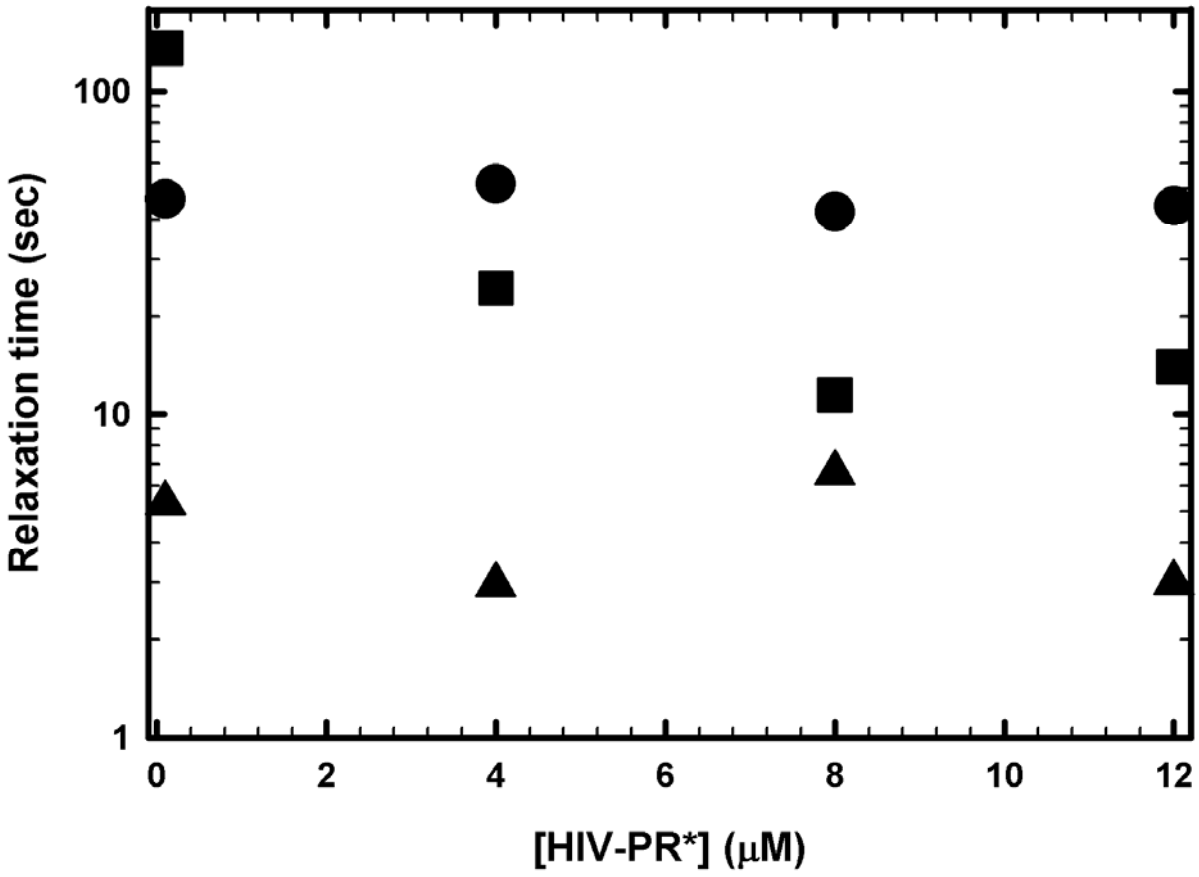
Figure S4. Predicted time constants from an eigenvalue analysis at a series of protein concentrations using the parameters from the global fit are overlaid on the chevron from Figure 5. The bimolecular reaction was approximated as a pseudo-first order reaction to calculate the eigenvalues, and therefore the plot is intended to semi-quantitatively show predicted dependence of time constants on the final urea concentration. Predicted time constants at a final protein concentration of 0.1 μM (blue), 1 μM (green) and 10 μM (red) are shown.



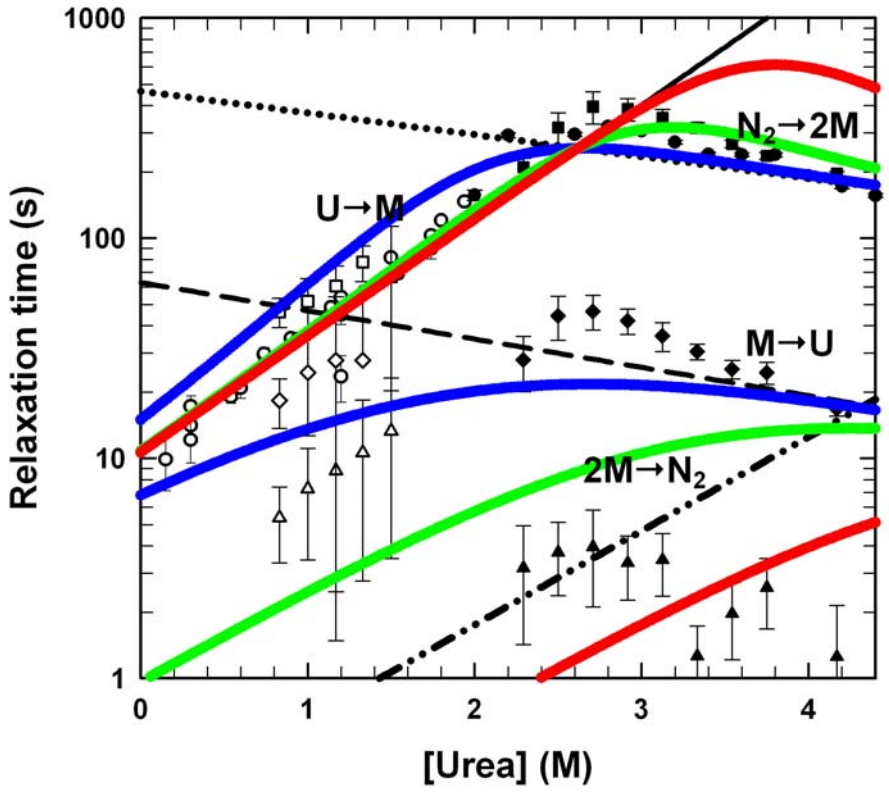
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4