Supporting Information for:

Binding of clinical inhibitors to a model precursor of a rationally selected multidrug resistant HIV-1 protease is significantly weaker than that to the released mature enzyme

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# Equally contributed to the experimental work

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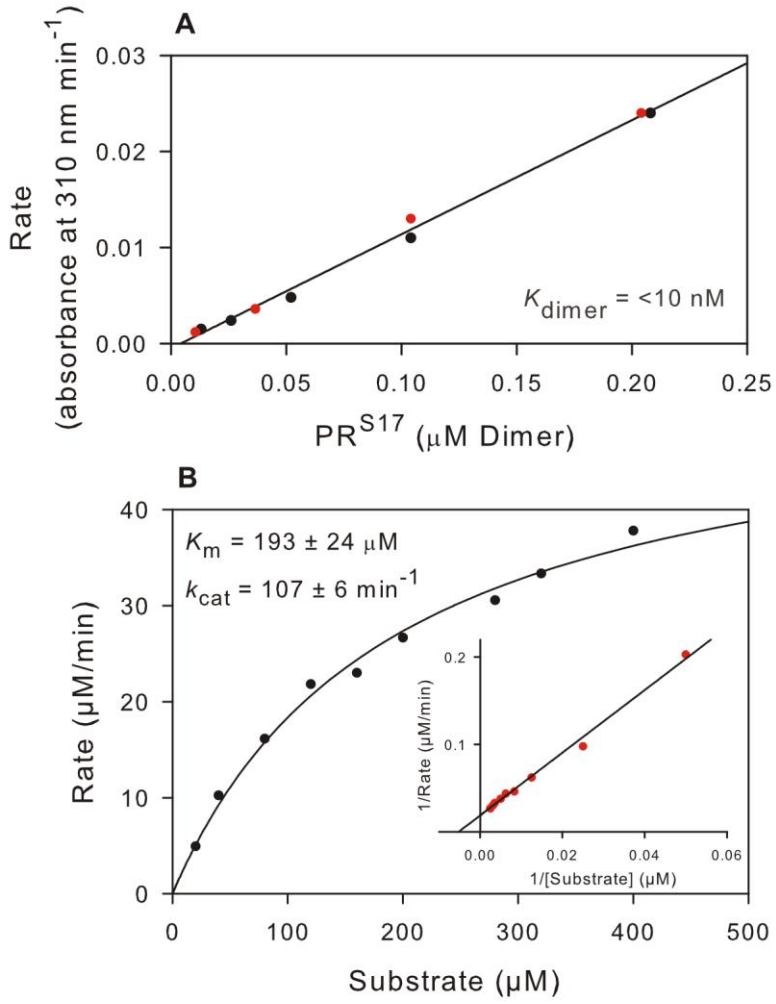
Table S1. Selected mutations in PR20[1,2] compared with PR^{S17} and PR22[3], and their predicted resistance to clinical PIs. The color scheme used for the inhibitors corresponds to the predicted significance of each substitution mutation (columns 2, 4 and 6) to impact resistance to each drug (columns 3, 5 and 7), in decreasing order, as bold red > bold black > plain black. Mutations in PR^{S17} and PR22 matching those in PR20 are in red and conservative substitutions in PR^{S17} and PR22 relative to PR20 are in blue. Dashes indicate residues identical to the wild type.

Residue (wild-type)	Mutations in PR20	Associated Drug Resistance	Mutations in PR^{S17}	Associated Drug Resistance	Mutations in PR22	Associated Drug Resistance
T4					S	Undefined
L10	F	APV, ATV, LPV, NFV[b]	I	APV, ATV, LPV, NFV, IDV, SQV[b]	V	APV, ATV, LPV, IDV, SQV[b]
I13	V	Undefined	-		A	Undefined
K14	-		-		R	Undefined
I15	V	Undefined	-		-	
K20	-		R	ATV, LPV, IDV[b]	I	ATV[b]
A22	-		-		V	Undefined
D30	N	**NFV**[a]	-		-	
V32	I	**APV**, ATV, **DRV**, **LPV**, **IDV**[a]	-		-	
L33	F	**APV**, ATV, DRV, LPV, NFV[a]	-		I	ATV[b]
E35	D	Undefined	D	Undefined	D	Undefined
M36	I	ATV, NFV, IDV[b]	I	ATV, NFV, IDV[b]	I	ATV, NFV, IDV[b]
S37	N	Undefined	D	Undefined	D	Undefined
R41	-		-		K	Undefined
K43	-		-		S	Undefined
M46	-		L	**APV**, ATV, LPV, **NFV**, **IDV**[a]	-	
I47	V	**APV**, ATV, **DRV**, LPV, NFV, IDV[a]	-		-	
G48	-		V	**ATV**, LPV, **NFV**, **SQV**[a]	A	Undefined
I54	L	**APV**, ATV, **DRV**, **LPV**, **NFV**, IDV, SQV[a]	V	APV, ATV, **LPV**, **NFV**, **IDV**, **SQV**[a]	V	APV, ATV, **LPV**, **NFV**, **IDV**, **SQV**[a]
Q58	E	TPV	-		-	
D60	-		E	ATV[b]	-	
I62	V	ATV, SQV[b]	V	ATV, SQV[b]	-	
L63	P	LPV[b]	P	LPV[b]	-	
I66	-		-		F	Undefined
H69	-				K	TPV[b]
A71	V	ATV, LPV, NFV, IDV, SQV[b]	V	ATV, LPV, NFV, IDV, SQV[b]	-	
I72	-		V	Undefined	-	
T74	-		-		S	Undefined
V77	-		I	NFV, IDV, SQV[b]	-	
V82	-		S	APV, ATV, **LPV**, **NFV**, **IDV**[a]	A	APV, ATV, **LPV**, **NFV**, **IDV**, SQV[a]

I84	V	**APV**, **ATV**, DRV, **LPV**, **NFV**, **IDV**, **SQV**[a]	-		V	**APV**, **ATV**, DRV, **LPV**, **NFV**, **IDV**, **SQV**[a]
N88	D	**NFV** (together with D30N)[a]	-		-	
L89	T	Undefined	-		I	TPV[b]
L90	M	**APV**, **ATV**, LPV, **NFV**, **IDV**, **SQV**[a]	M	**APV**, **ATV**, LPV, **NFV**, **IDV**, **SQV**[a]	M	**APV**, **ATV**, LPV, **NFV**, **IDV**, **SQV**[a]
T91	-		-		S	Undefined
I93	-		L	ATV[b]	-	

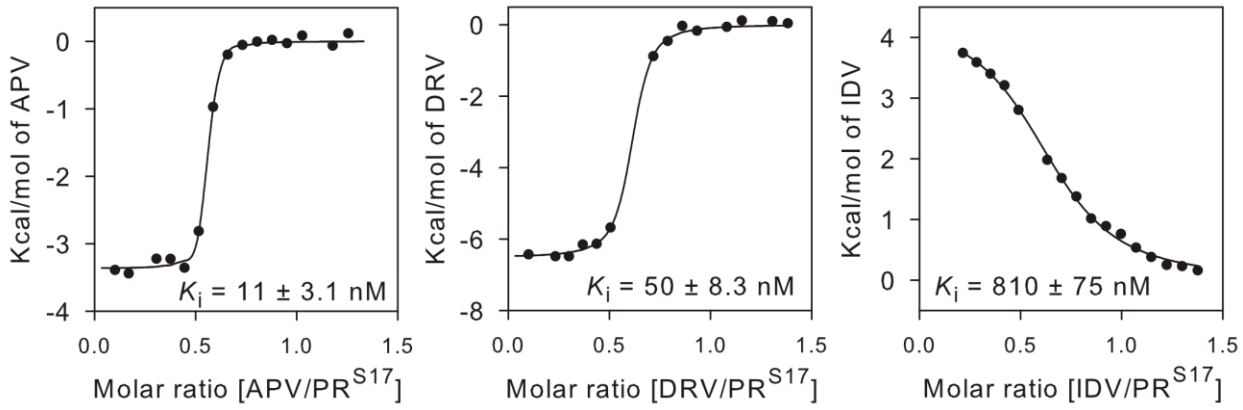
Drug resistance data were compiled from [a]Stanford University drug resistance database (http://hivdb.stanford.edu/DR/PIResiNote.html) and [b]Wensing A. M. et al.[4]

Figure S1



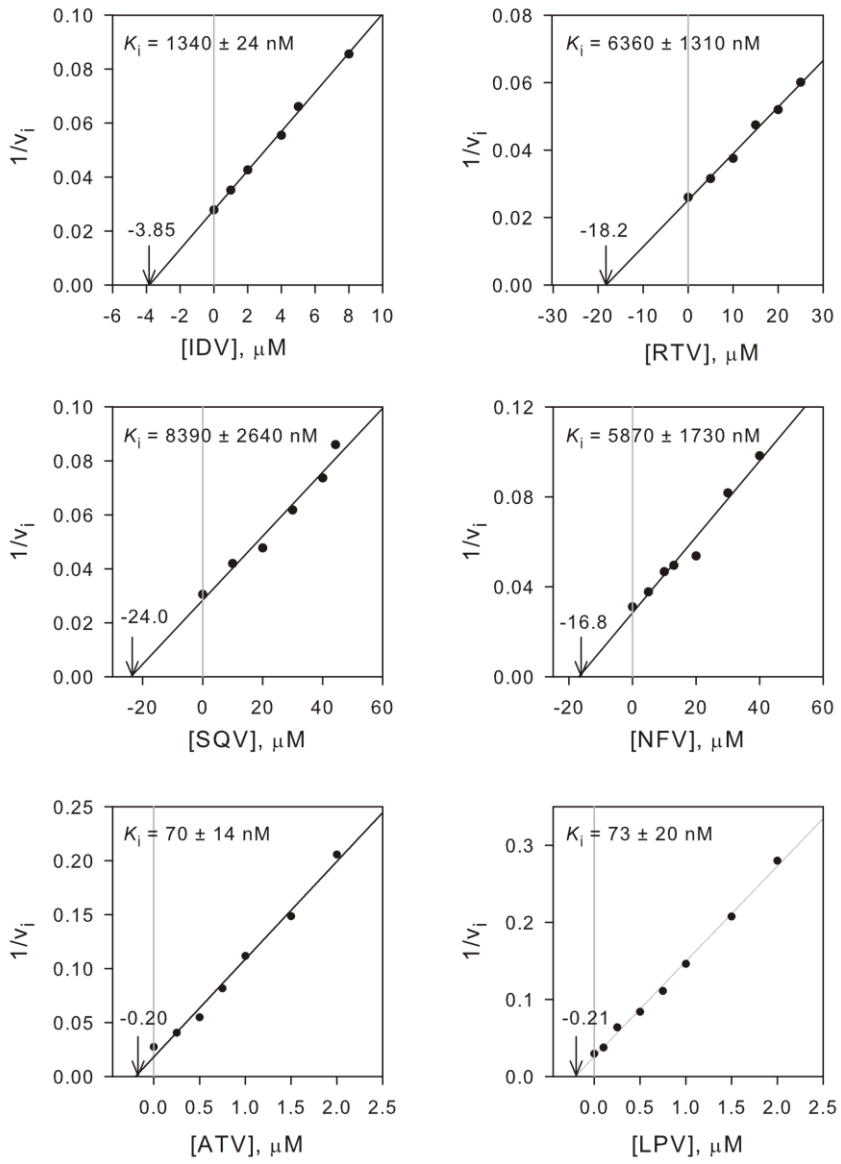
Dimer dissociation constant (K_{dimer}) and kinetic parameters measured for PR^{S17} in 50 mM sodium acetate, pH 5, containing 250 mM NaCl (buffer B) at 28 °C. (A) Dependence of the initial rate of hydrolysis of the chromogenic substrate on PR^{S17} concentration. Red and black symbols denote duplicate experiments. (B) Michaelis-Menten and Lineweaver-Burk (inset) plots for hydrolysis of the chromogenic substrate catalyzed by 0.5 μM PR^{S17} (as dimer) in buffer B. Kinetic parameters K_m and k_{cat} were determined by curve fitting using the enzyme kinetics module of SigmaPlot 10.

Figure S2



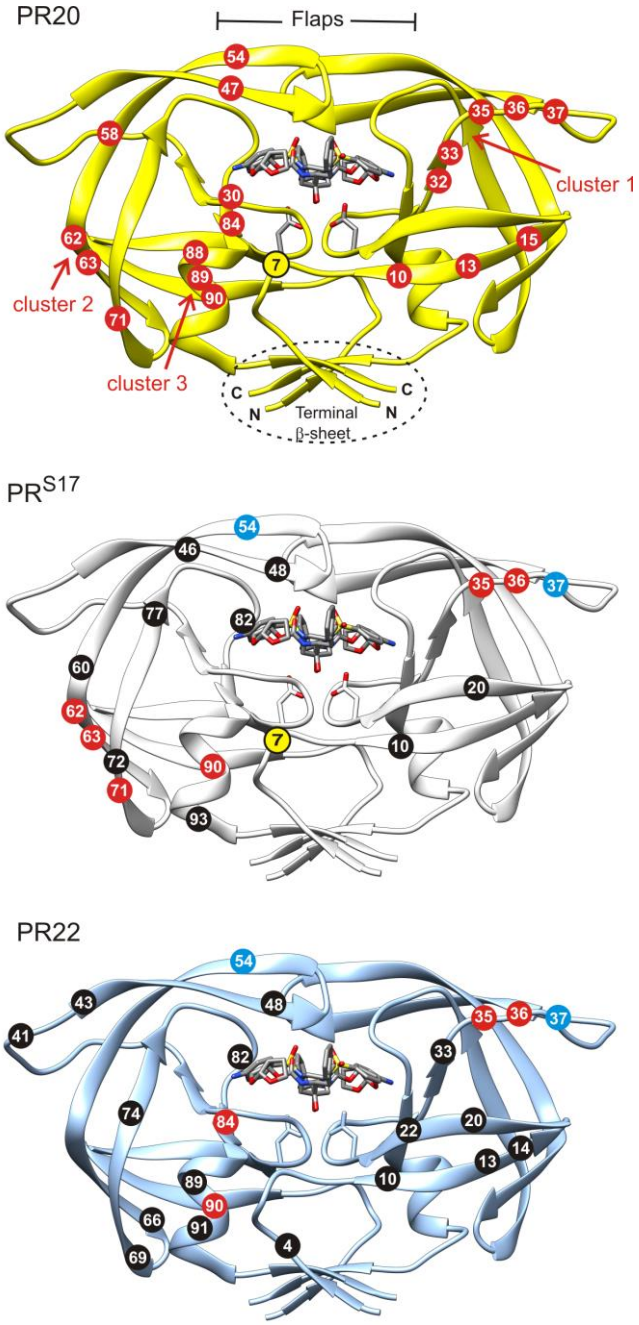
K_i determination for the binding of APV, DRV and IDV to PR^{S17} by ITC in buffer A (50 mM sodium acetate, pH 5) at 28 °C. The values shown correspond to $1/K_{association}$ derived by curve fitting using Origin ITC software.

Figure S3



Kinetic determination of IC_{50} and K_i for the binding of selected inhibitors to mature PR^{S17} in buffer B (50 mM sodium acetate, 250 mM NaCl, pH 5) at 28 °C. Arrows indicate IC_{50}. K_i values (shown in Table 1) were calculated from the relationship $K_i = IC_{50}/(1 + [substrate]/K_m)$.

Figure S4



Comparison of mutations of PR^{S17} with PR20[1,2] and PR22.[3] Ribbon representation (3UCB[5]) showing location of 19 (top), 17 (middle) and 22 (bottom) mutations in PR20, PR^{S17} and PR22, respectively. Red and blue residue positions in PR^{S17} and PR22 denote identical and conservative substitutions, respectively, matching those in PR20. Yellow (Q7K) substitution is introduced in PR20 and PR^{S17} constructs to restrict autoproteolysis (self-degradation).

REFERENCES

1. Dierynck, I., De Wit, M., Gustin, E., Keuleers, I., Vandersmissen, J., Hallenberger, S., and Hertogs, K. (2007) Binding kinetics of darunavir to human immunodeficiency virus type 1 protease explain the potent antiviral activity and high genetic barrier. *J. Virol. 81*, 13845-13851.

2. Louis, J. M., Aniana, A., Weber, I. T., and Sayer, J. M. (2011) Inhibition of autoprocessing of natural variants and multidrug resistant mutant precursors of HIV-1 protease by clinical inhibitors. *Proc. Natl. Acad. Sci. U. S. A 108*, 9072-9077.

3. Kozisek, M., Henke, S., Saskova, K. G., Jacobs, G. B., Schuch, A., Buchholz, B., Muller, V., Krausslich, H. G., Rezacova, P., Konvalinka, J., and Bodem, J. (2012) Mutations in HIV-1 gag and pol compensate for the loss of viral fitness caused by a highly mutated protease. *Antimicrob. Agents Chemother. 56*, 4320-4330.

4. Wensing, A. M., Calvez, V., Gunthard, H. F., Johnson, V. A., Paredes, R., Pillay, D., Shafer, R. W., and Richman, D. D. (2014) 2014 Update of the drug resistance mutations in HIV-1. *Top. Antivir. Med. 22*, 642-650.

5. Agniswamy, J., Shen, C. H., Aniana, A., Sayer, J. M., Louis, J. M., and Weber, I. T. (2012) HIV-1 protease with 20 mutations exhibits extreme resistance to clinical inhibitors through coordinated structural rearrangements. *Biochemistry 51*, 2819-2828.