

Supplementary material

Table S1: The names of the isolates, whose RF data was used in this study, as reported in HIVdb, and the reference of the study where RF measurements were performed.

Mutation		Isolate	Reference
M46I	wildtype	71V-11, A71V-7, Bru-A71V-3	[1]
	mutant	P372	[2]
I50L	wildtype	71V-11, A71V-7, Bru-A71V-3	[1]
	mutant	71V-12, A71V-8, BruA71V-4	[1]
I84V	wildtype	JGP-M1C	[3]
	mutant	JGP-M2C, JGP-M2R	[3]
N88S	wildtype	RZ27 (IDV), RZ28 (FPV)	[4]
	mutant	RZ22 (IDV), RZ-L4 (FPV)	[4]

Table S2: Inhibitor binding free energy change upon switching the proton from the reference protonated active site residue to the active site residue on the opposite subunit for wildtype and mutant proteins. $\pm$ shows bootstrap error estimate, all values in kcal/mol.

Inhibitor	Genotype	Reference protonated state	$\Delta\Delta G_{WT}^{prot}$	$\Delta\Delta G_{MUT}^{prot}$
APV	M46I	D25'	-1.86 ± 0.23	-2.32 ± 0.21
IDV	M46I	D25	1.15 ± 0.27	0.53 ± 0.45
APV	I50L	D25'	-1.74 ± 0.31	-1.85 ± 0.18
IDV	I50L	D25	1.64 ± 0.23	0.52 ± 0.38
APV	I84V	D25'	-1.67 ± 0.25	-0.31 ± 0.27
IDV	I84V	D25	1.67 ± 0.44	1.29 ± 0.38
LPV	I84V	D25'	-0.6 ± 0.35	-1.33 ± 0.3
SQV	I84V	D25	1.03 ± 0.19	2.04 ± 0.27
APV	N88S	D25	2.23 ± 0.29	-0.16 ± 0.24
IDV	N88S	D25	2.22 ± 0.51	1.42 ± 0.45

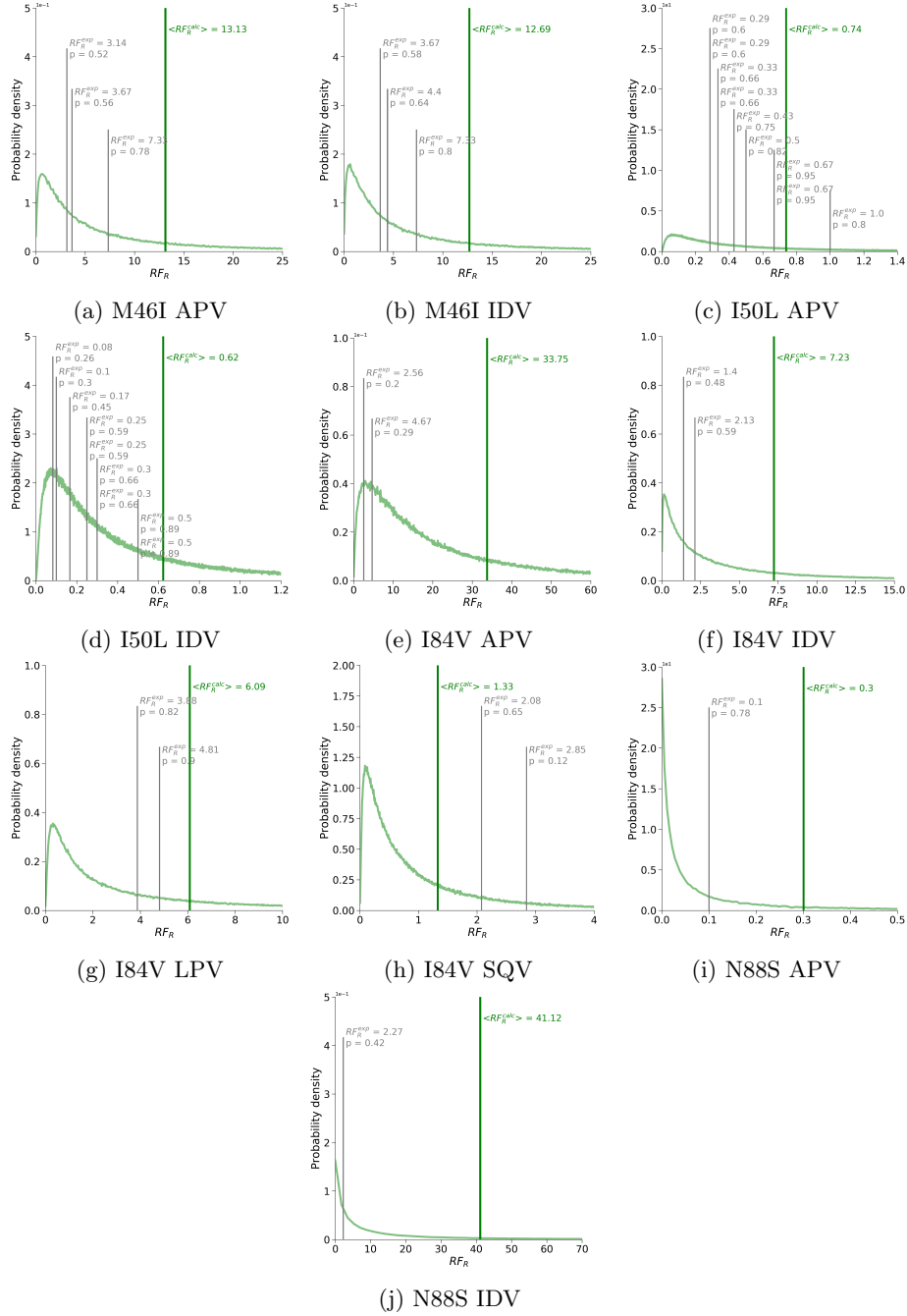


Figure S1: Calculated RF_R distributions and experimental estimates. p designates the proportion of RF_R^{calc} at least as extreme as RF_R^{exp} compared to mean RF_R^{calc} . *Nota bene*: in case of APV, RF_R^{exp} measurements are for its prodrug FPV.

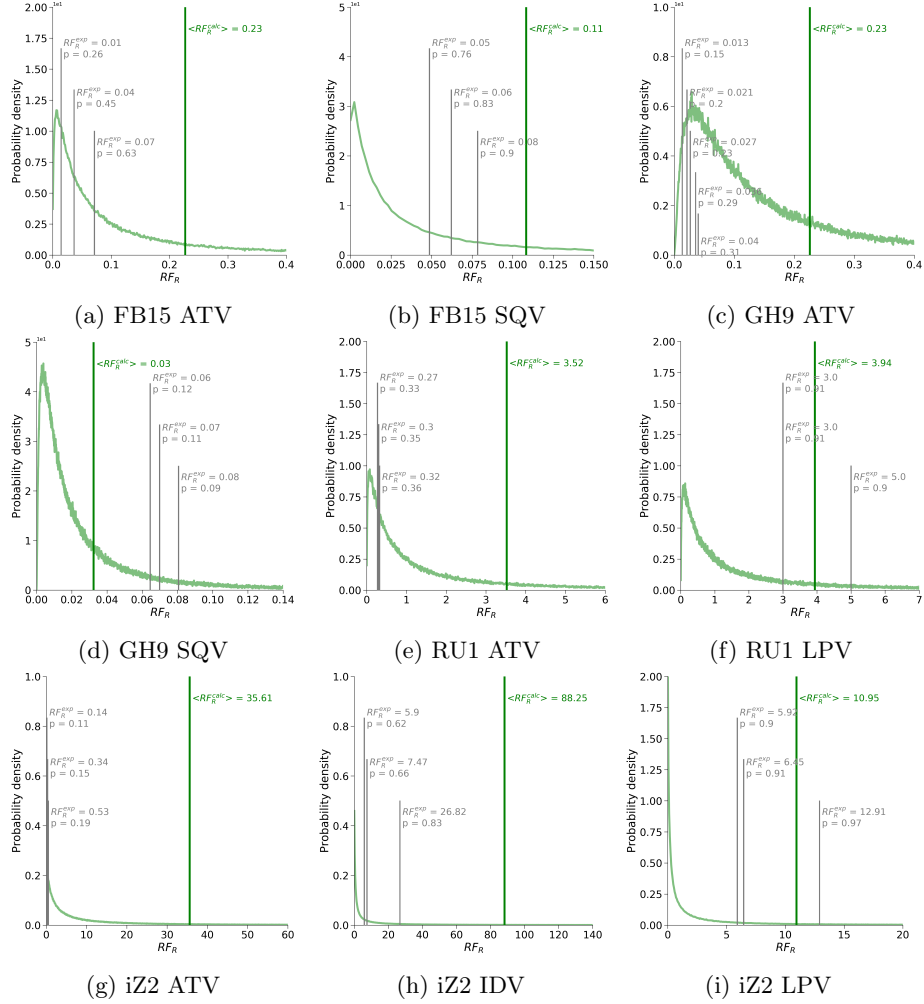


Figure S2: Calculated RF_R distributions and experimental estimates. p designates the proportion of RF_R^{calc} at least as extreme as RF_R^{exp} compared to mean RF_R^{calc} . *Nota bene:* in case of APV, RF_R^{exp} measurements are for its produg FPV.

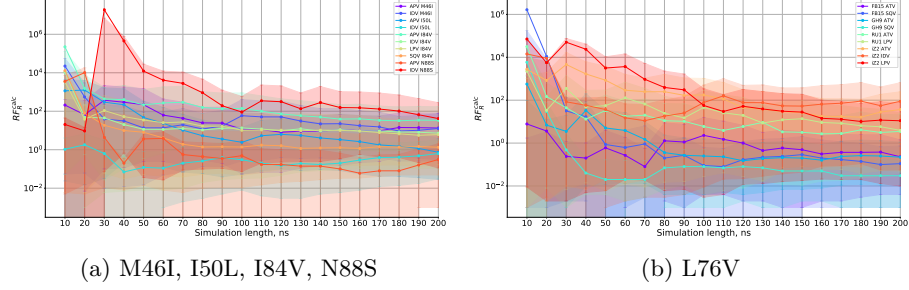


Figure S3: Convergence of the RF_R estimates. The shaded areas show the 95% credible interval.

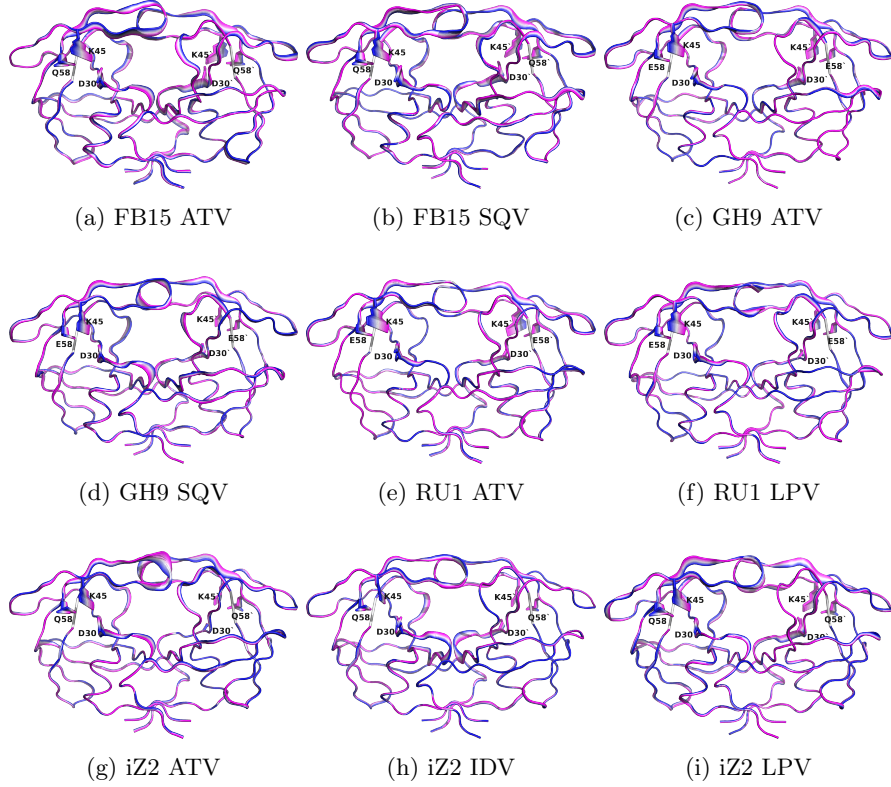


Figure S4: Interpolation between the extremes of the FMA models for the corresponding complexes. Blue-to-magenta bands correspond to the interpolation along the mode as represented as cartoon for backbone and as sticks for residues 30, 45, and 58, with blue corresponding to L76 state and magenta to V76 state. Mutated residue 76 is not part of the model and is represented here as gray dash.

Table S3: Average hydrogen bonds number between residues D30, T31, and T74 with N88 and S88 for wildtype and mutant complexes, respectively. Columns 3 and 4 of the table corresponds to hydrogen bonds within monomer A of protease and columns 5 and 6 of the table corresponds to hydrogen bonds within monomer B of protease (residues marked with prime symbol). $\pm$ indicates standard error of bond frequency across independent simulations.

Residues	Inhibitor	N88	S88	N88'	S88'
D30/D30'	APV	$0.005 \pm 2 \times 10^{-4}$	0.61 ± 0.05	$5 \times 10^{-4} \pm 3 \times 10^{-7}$	0.66 ± 0.12
	IDV	$2 \times 10^{-4} \pm 2 \times 10^{-7}$	0.22 ± 0.008	$0.001 \pm 2 \times 10^{-6}$	0.55 ± 0.08
T31/T31'	APV	1.28 ± 0.004	0.21 ± 0.03	1.56 ± 0.003	0.13 ± 0.04
	IDV	1.26 ± 0.007	0.47 ± 0.03	1.35 ± 0.008	0.27 ± 0.04
T74/T74'	APV	0.8 ± 0.003	$3 \times 10^{-4} \pm 2 \times 10^{-7}$	0.85 ± 0.001	$2 \times 10^{-4} \pm 2 \times 10^{-7}$
	IDV	0.71 ± 0.001	$2 \times 10^{-4} \pm 2 \times 10^{-7}$	0.79 ± 0.001	$3 \times 10^{-4} \pm 9 \times 10^{-7}$

Table S4: Inhibitor binding free energy change upon switching the proton from the reference protonated active site residue to the active site residue on the opposite subunit for wildtype and mutant proteins. $\pm$ shows bootstrap error estimate, all values in kcal/mol.

Inhibitor	Genotype	Reference protonated state	$\Delta\Delta G_{WT}^{prot}$	$\Delta\Delta G_{MUT}^{prot}$
ATV	FB15	D25'	-1.17 ± 0.31	-2.08 ± 0.36
SQV	FB15	D25	-0.08 ± 0.25	0.4 ± 0.37
ATV	GH9	D25'	-0.13 ± 0.21	-1.23 ± 0.32
SQV	GH9	D25	0.45 ± 0.21	-0.08 ± 0.31
ATV	RU1	D25'	-4.21 ± 0.38	-1.76 ± 0.47
LPV	RU1	D25'	-1.41 ± 0.32	-0.75 ± 0.37
ATV	iZ2	D25	-0.82 ± 0.34	-1.25 ± 0.4
IDV	iZ2	D25'	-0.95 ± 0.44	1.43 ± 0.31
LPV	iZ2	D25	0.56 ± 0.27	0.5 ± 0.75

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