Supplementary Materials

for

Structural basis for drug and substrate specificity exhibited by FIV encoding a chimeric FIV/HIV protease

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Table S1 **Crystallographic statistics for 6s-98S FIV PR complexes**		
Complex	**Darunavir**	**Lopinavir**
PDB code	3OGP	3OGQ
Space group	$P3_1$	$P3_1$
Unit cell dimensions (Å)	81.17 81.17 33.59	81.79 81.79 33.94
PR dimers per asymmetric unit	1	1
Solvent content	51.9%	53.1%
Data		
Total observations > $0\sigma_F$	76,944	78,492
Unique reflections > $0\sigma_F$	27,120	21,978
Redundancy	2.8 (2.7)	3.6 (3.3)
Completeness	99.5% (97.1%)	93.5% (84.9%)
Resolution (last shell) (Å)	27.0 – 1.70 (1.79 – 1.70)	70.8 – 1.80 (1.90 – 1.80)
$<I/\sigma_I>$ all data (last shell)	12.8 (2.2)	10.9 (2.0)
Rmerge all data (last shell)	0.051 (0.346)	0.051 (0.401)
Refinement		
R-factor	0.185	0.225
Rfree	0.228	0.279
Reflections used	25,748	20,791
Test set	1,358 (5.0%)	1,186 (5.4%)
RMSD from ideality		
Bond lengths (Å)	0.009	0.012
Bond angles (deg.)	1.29	1.41
Ramachandran plot		
Favored regions	94.5%	94.1%
Allowed regions	99.1%	98.6%
Model		
Monomer A	Residues / Avg. B ($Å^2$)	Residues / Avg. B ($Å^2$)
Protein	112 (24.1)	112 (17.4)
Drug (occupancy 0.50)	1 (15.1)	1 (17.3)
Monomer B		
Protein	112 (24.1)	112 (17.3)
Drug (occupancy 0.50)	1 (14.9)	1 (16.8)
H_2O molecules	158 (26.9)	144 (22.0)
DMSO	1 (33.9)	2 (41.5)

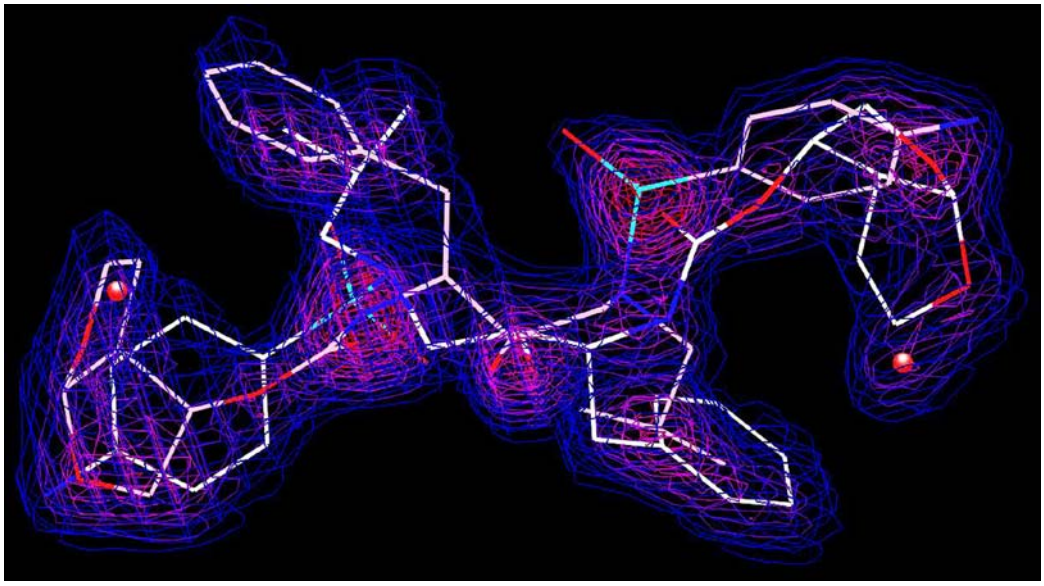

Figure S1

Unbiased, σ_A weighted 2|Fo|-|Fc| electron density at 1.7 Å resolution for darunavir in 6s-98S FIV PR contoured at 1, 2, 3, 4 and 5σ. The inhibitor is disordered about the local 2-fold axis of the PR dimer, with 0.5 occupancy in monomers A and B. Red spheres are H_2O molecules present at 0.5 occupancy.

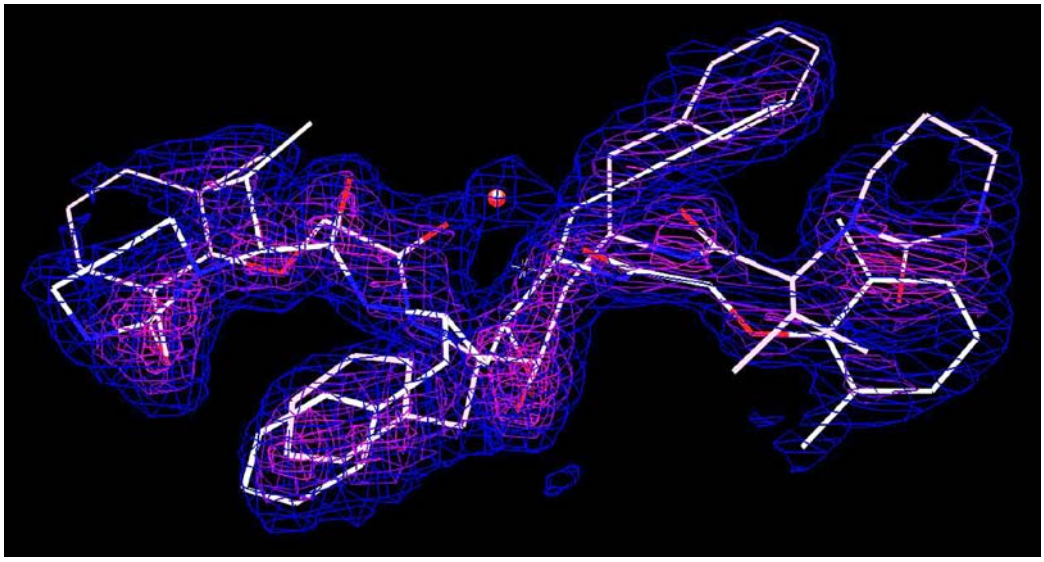

Figure S2

Unbiased, σ_A weighted 2|Fo|-|Fc| electron density at 1.8 Å resolution for lopinavir in 6s-98S FIV PR contoured at 1, 2, 3, and 4σ. The inhibitor is disordered about the local 2-fold axis of the PR dimer, with 0.5 occupancy in monomers A and B. The red sphere is a H_2O molecule (i.e. the 'flap' water, no. 202) present at 0.5 occupancy, which interacts with the amides of Ile59 in each flap.

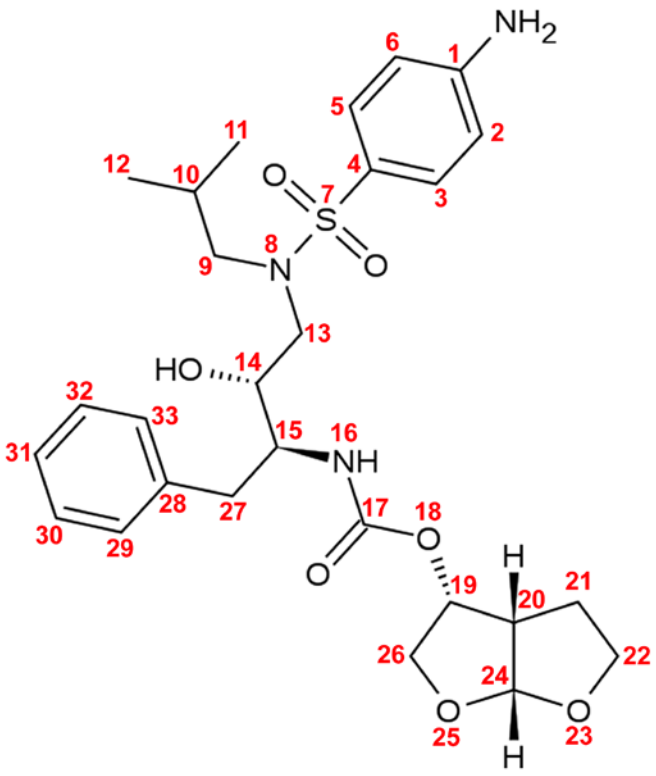

Figure S3
Atom numbering for darunavir (DRV)
as in Table S2

Table S2
Hydrogen bond and electrostatic interactions of DRV with w.t. HIV PR (2IEN) and 6s-98S FIV PR for monomers A and B

DRV interaction [A]	w.t. HIV DRVa [B]	w.t. HIV DRVb	6s-98S FIV [C] DRVa	6s-98S FIV DRVb
HB [D] with NH2 at 1	D30 O 3.3 Å 143º	D30 O 3.2 Å 143º	I35 [30] O 3.2 Å 144º	I35 [30] O 3.5 Å 162º
HB with NH2 at 1	D30 COO^- 2.7 Å 132º	D30 COO^- 2.8 Å 137º	(I35 [30])	(I35 [30])
HB with NH2 at 1				H_2O 434 3.1 Å 162º
HB with O at 7	H_2O 1068 [F] 2.4 Å 147º	H_2O 1068 2.4 Å 132º		V59I [50] NH 3.0 Å 154º
HB with OH at 14	D25 COO^- 2.8 Å 165º	D25 COO^- 2.8 Å 166º	D30 [25] COO^- 2.9 Å 135º	D30 [25] COO^- 2.7 Å 161º
HB with NH at 16	G27 O 3.2 Å 156º	G27 O 3.2 Å 158º	G32 [27] O 3.1 Å 163º	G32 [27] O 3.0 Å 159º
Elec [E] with NH at 16	D25 COO^- 4.4 Å 92º	D25 COO^- 4.4 Å 90º	D30 [25] COO^- 5.2 Å 94º	D30 [25] COO^- 4.2 Å 90º
HB with O at 17	H_2O 1068 3.0 Å 165º	H_2O 1068 3.0 Å 179º		
Elec with O at 18	D29 NH 4.6 Å 136º	D29 NH 4.5 Å 135º	D34 [29] NH 4.7 Å 127º	D34 [29] NH 4.7 Å 127º
HB with O at 23	D29 NH 3.0 Å 157º	D29 NH 2.9 Å 159º	D34 [29] NH 3.0 Å 173º	D34 [29] NH 3.1 Å 163º
Elec with O at 23	D30 NH 4.5 Å 137º	D30 NH 4.5 Å 139º	I35 [30] NH 4.7 Å 144º	I35 [30] NH 4.5 Å 144º
Elec with O at 23	R8 side-chain 4.0 Å 118º	R8 side-chain 4.1 Å 128º	R13 [8] side-chain 4.4 Å 127º	R13 [8] side-chain 4.6 Å 139º
HB with O at 25	D30 NH 3.3 Å 158º	D30 NH 3.3 Å 164º	I35 [30] NH 3.2 Å 178º	
HB with O at 25	D29 NH 3.1 Å 119º	D29 NH 3.0 Å 117º	D34 [29] NH 3.1 Å 112º	D34 [29] NH 3.2 Å 113º
Elec with O at 25			I35 [30] NH 3.2 Å 175º	I35 [30] NH 3.4 Å 173º

A. DRV atom numbering as in Fig. S3
B. Occupancy of DRV 0.55 and 0.45 in monomers A and B of the HIV complex, and 0.50 and 0.50 in monomers A and B of the 6s-98S PR complexes.
C. For FIV 6s-98S residues, the number in the superscript is the corresponding HIV residue.
D. HB – hydrogen bond: length in Angstroms; donor—H—acceptor angle in degrees.
E. Elec – favorable electrostatic interaction; corresponding donor—H—acceptor angle in degrees.
F. H_2O 1068 is the 'flap water'.

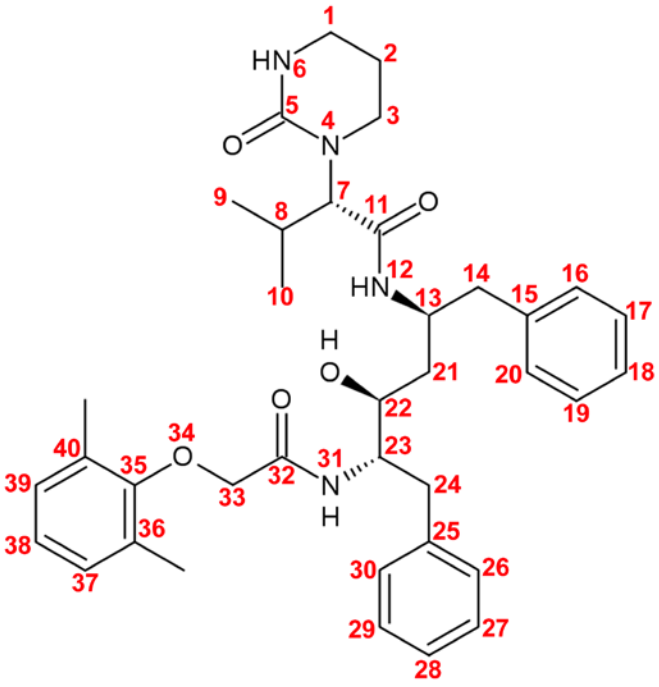

Figure S4
Atom numbering for lopinavir (LPV)
as in Table S3

Table S3
Hydrogen bond and electrostatic interactions of LPV with w.t. HIV PR (1MUI) and 6s-98S FIV PR for monomers A and B

LPV interaction [A]	w.t. HIV LPVa [B]	w.t. HIV LPVb	6s-98S FIV [C] LPVa	6s-98S FIV LPVb
HB [D] with O at 5	D29 NH 2.6 Å 159º	D29 NH 2.7 Å 165º	D34 [29] COO^- 3.2 Å 166º	D34 [29] COO^- 2.8 Å 162º
HB with O at 5			H_2O 404 3.1 Å 132º	
HB with NH at 6	D29 COO^- 3.2 Å 171º	D29 COO^- 2.4 Å 159º		
Elec [E] with NH at 6			D34 [29] COO^- 3.4 Å 137º	D34 [29] COO^- 3.0 Å 92º
HB with O at 11			H_2O 202 [F] 3.0 Å 118º	H_2O 202 3.4 Å 130º
HB with NH at 12	G27 O 3.3 Å 152º	G27 O 2.9 Å 149º		G32 [27] O 2.9 Å 159º
Elec with NH at 12	D25 COO^- 4.8 Å 105º	D25 COO^- 4.4 Å 111º	G32 [27] O 3.7 Å 114º	D30 [25] COO^- 4.9 Å 94º
HB with OH at 22	D25 COO^- 2.6 Å 174º	D25 COO^- 2.8 Å 172º		
Elec with OH at 22			D30 [25] COO^- 2.9 Å 93º	D30 [25] COO^- 2.8 Å 105º
Elec with OH at 22			G32 [27] O 3.9 Å 139º	G32 [27] O 3.4 Å 125º
HB with NH at 31			G32 [27] O 3.5 Å 158º	G32 [27] O 2.9 Å 144º
Elec with NH at 31	G27 O 3.7 Å 132º	G27 O 4.0 Å 130º		
Elec with NH at 31	D25 COO^- 4.1 Å 126º	D25 COO^- 4.4 Å 118º	D30 [25] COO^- 4.3 Å 100º	D30 [25] COO^- 4.2 Å 98º
Elec with O at 32	I50 NH 3.7 Å 110º	I50 NH 3.6 Å 142º	I59 [50] NH 4.3 Å 149º	I59 [50] NH 4.9 Å 156º

A. LPV atom numbering as in Fig. S4
B. Occupancy of LPV 0.5 in monmers A and B of both w.t. HIV and 6s-98S PR complexes.
C. For FIV 6s-98S residues, the number in the superscript is the corresponding HIV residue.
D. HB – hydrogen bond: length in Angstroms; donor—H—acceptor angle in degrees.
E. Elec – favorable electrostatic interaction; corresponding donor—H—acceptor angle in degrees.
F. H_2O 202 is the 'flap water'; no H_2O sites included in PDB deposition 1MUI.

Table S4
Hydrophobic contacts [1] of DRV and LPV with w.t. HIV PR [2] and 6s-98S FIV PR

w.t. HIV residue	6s-98S FIV residue	w.t. HIV / 6s-98S FIV			
		DRVa [3]	DRVb	LPVa [4]	LPVb
L23	L28	H / F	HH / F	/ F	/ FFFF
G27	G32	H /	H / F		
A28	A33	HH / FFF	HH / FF	/ FFFFF	H / FFFF
D29	D34	H /	H /	/ FFF	
D30	I35			H / FF	HHH / FF
V32	I37V	HH /	H / F	H / F	H /
I47	M56	/ FFFF	/ F	/ F	HH / FFFF
G48	I57			H / FF	/ FF
G49	G58	HHH / FFFF	HHH / F	H /	H / FF
I50	V59I	HH / FF	HH / FF	HH / FFF	HHH / FF
P81	I98S	H /	HHH /	HHHHH /	HHHHHHHHHH /
V82	Q99V	HHHH / F	H / F	/	HH / F
I84	L101	HH / FFF	HH / FFF	HH / FF	HHH / FF

1. Hydrophobic packing interactions measured by RCSB PDB Ligand Explorer 3.8 using C-C distances less than or equal to 3.9 Å. H = hydrophobic packing contact present with HIV PR. **F** = hydrophobic packing contact present with 6s-98S FIV PR. **F** = hydrophobic packing contact present with one of the 6 mutated residues in 6s-98S FIV PR.
2. HIV PR structures with DRV bound (PDB 2IEN) and with LPV bound (PDB 1MUI).
3. Occupancy of DRV 0.55 and 0.45 in monomers A and B of the HIV complex, and 0.50 and 0.50 in monomers A and B of the 6s-98S PR complexes.
4. Occupancy of LPV 0.5 in monomers A and B of both w.t. HIV and 6s-98S PR complexes.