Supporting Information

Second binding site of DRV and SQV

A second inhibitor molecule is seen bound to PR20/DRV and PR20/SQV dimers in addition to the usual inhibitor bound in the active site cavity. In PR20/DRV complex, the second DRV with 0.6 occupancy is bound in the same site on the flap as described previously for the single mutant V32I/DRV complex.[1] The only structural difference in the second DRV of PR20/DRV complex occurs at the P1 phenylalanine moiety, which cannot interact with residue 35′ due the mutation of E35′D to a shorter side chain, in addition to the buckling of the hinge loop due to M36′I mutation. Instead, the P1 group realigns to form three van der Waals contacts with Pro79′.

In the PR20/SQV complex, the second SQV-2 binds in a site adjacent to the usual active site SQV-1 (Figure S2B), which is different from that of the second DRV. The SQV-2 molecule occurs in a bent conformation such that P2′ and P3 groups form van der Waals contacts with P1′ of SQV-1 bound at the active site. The P3 residue also has extensive van der Waals interactions with the inter-monomer ion pair residues Arg8 and Asp29′, while the P2 group forms a hydrogen bond with Arg8 and van der Waals contacts with L10F. The P2′ residue of SQV has hydrophobic contacts with residues Pro81 and Val82. The bent conformation enables the P1 group of SQV-2 to form van der Waals contacts with the P1 and P3 groups of SQV-1 in the active site of a symmetry related molecule. The hydroxyl group forms a hydrogen bond to the carbonyl oxygen of Pro81. Thus, there is a continuous lattice alternating the extended conformation SQV-1 bound at the active site and SQV-2 in a bent conformation. A second SQV was seen at the same site in the recent crystal structure of PR triple mutant (V32I, I47V and V82I) in complex with SQV albeit in extended conformation.[2]

References

(1) Kovalevsky, A. Y., Liu, F., Leshchenko, S., Ghosh, A. K., Louis, J. M., Harrison, R. W., and Weber, I. T. (2006) Ultra-high resolution crystal structure of HIV-1 protease mutant reveals two binding sites for clinical inhibitor TMC114. *J Mol Biol 363*, 161-173.

(2) Tie, Y., Wang, Y.F., Boross, P.I., Chiu, T.Y., Ghosh, A.K., Tozser, J., Louis, J.M., Harrison, R.W. and Weber, I.T. (2011) Critical differences in HIV-1 and HIV-2 protease specificity for clinical inhibitors. *Protein Sci 21*, 339-350.

Supporting Information Figures

Figure S1: A. Residue B-factors in PR20 structures showing different flap conformations. The cartoon diagram of the fully open flaps in the yttrium complex, partially open and closed conformation flaps in the structure of PR20/p2-NC and fully closed flaps in PR20/DRV are colored by atomic displacement parameter (ADP) spectrum in PyMOL. Dark blue and dark red represent the lowest and highest ADP values. The spectrum range is 16-66, 15-89 and 11-94 $Å^2$ for inhibitor free PR20, PR20/p2-NC and PR20/DRV, respectively. The inhibitors were omitted for clarity. B. The open and closed PR20 dimers present in one asymmetric unit of the PR20/p2-NC crystal structure are shown in red and cyan cartoons. The p2-NC peptide analog found only in the cyan dimer is represented in magenta sticks. C. F_o-F_c omit map (red) of the major conformation of SQV (green sticks) bound at the active site contoured at 3σ level. D. F_o-F_c omit map of the p2-NC substrate analog bound at the active site contoured at 2σ level and colored as in S1C.

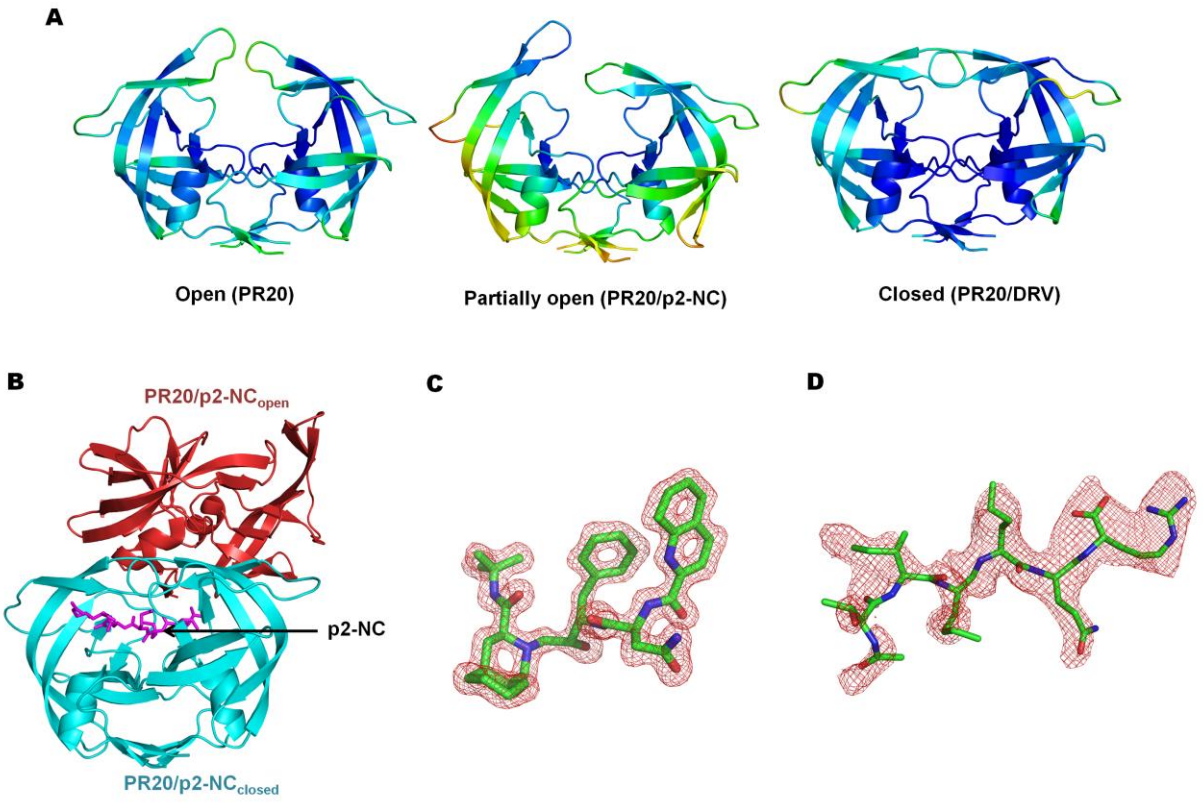

Figure S2: A. The active site cavity of PR20/p2-NC_{open} dimer (red cartoon) in one asymmetric unit is occupied by the two flaps from a symmetry related PR20/p2-NC_{open} dimer (blue cartoon). The interactions with symmetry related flapB twist the tip of the closed flapB. The active site aspartates are represented as green sticks. B. The PR20/SQV complex is shown as yellow cartoons with SQV-1 (magenta sticks) bound at the usual active site location interacting with the second SQV-2 (in orange carbons). The second SQV-2 binds in a different location to the second DRV binding to one flap of the PR20/DRV dimer. SQV-1 bound in the active site interacts with SQV-2 at one end as well as a symmetry related molecule of SQV-2 at the other end of the molecule producing continuous interactions throughout the crystal lattice. SQV-2 is in a bent conformation in comparison to the active site bound SQV-1.