Online Supporting Information

Extreme Entropy-Enthalpy Compensation in a Drug Resistant Variant of HIV-1 Protease

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Structure solution and crystallographic refinement. The suite of programs from CCP4i (*1*) was used for most of the crystallographic operations. Structure solution for all complexes was carried out using the molecular replacement package AMoRe (*2*). The complex of peptide fragment representing the Gag substrate capsid-p2 bound with D25N inactive HIV-1 protease (*3*) (PDB code: 1F7A) was used as the starting model. This substrate structure was used to solve inhibitor complexes to reduce model bias. For the orthorhombic crystals, the structure solution was straightforward. However, for the APV_{Flap+} and DRV_{Flap+} complexes, which crystallized in a hexagonal form, a combination of self-rotation maps were computed, revealing that the space group is $P6_1$ with two dimers per asymmetric unit. The subsequent refinement strategy involving ARP/wARP, TLS parameters and Refmac5 was similar to our earlier structural analyses (*4*). Interactive model building was conducted using the package O (*5*) and the quality of the structures was assessed using PROCHECK (*6*). The dimers of the APV_{Flap+} and DRV_{Flap+} complexes within the asymmetric unit were restrained by non-crystallographic symmetry (NCS) restraints. However, no NCS restraints were imposed between the monomers of the dimer for any of the complexes. The refinement statistics are provided in Table 2 of main manuscript.

Crystallographic waters and entropy-enthalpy compensation. The entropy change of binding is related to configurational entropy of inhibitor, enzyme, and also the water molecules. Due to the hydrophobic effect, water molecules are more ordered near exposed nonpolar surfaces. The loss of ordered water molecules upon binding an inhibitor gives rise to a favorable change in entropy, however also causes a loss in enthalpy as specific hydrogen bonds with and possibly within water molecules are broken. Crystallographic water molecules in Flap+ complexes were analyzed to assess their possible role in the observed entropy-enthalpy compensation. This

compensation is most pronounced for the binding of APV and DRV to Flap+ compared to WT, ($\Delta\Delta H_{APV}$ =10.7 kcal·mol^{-1}and 14.1 kcal·mol^{-1}, respectively) (Table 1 and Figure 2 of main manuscript). The approximate energy of a hydrogen bond (5 kcal·mol^{-1}) (*7*), could potentially account for the release of two to four water molecules for APV and DRV binding to Flap+. However, no systematic changes are present in the crystallographic waters within a 4.2 Å hydration shell around the protein, when Flap+ complexes are compared to those of WT and Act. Hence, any possible role of water structure in the entropy-enthalpy compensation is not apparent from the crystal structures.

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