

## Dimer disruption and monomer sequestration of HIV-1 protease by rationally designed interface peptidomimetics: mechanistic proofs

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The disruption of protein-protein interfaces by small molecules is a very promising strategy to inhibit functional protein complexes with huge therapeutical potential<sup>1</sup>. We designed molecules targeting specifically intermolecular beta-sheets. Such interactions have been identified in more than 2500 protein complexes. As an application, we developed inhibitors targeting the dimeric HIV-1 protease interface instead of the active site as do HIV-1 protease inhibitors (PIs) presently used in therapeutics. The use of PIs led to the occurrence of mutations in HIV-1 protease that are responsible for multi-drug cross-resistance. The highly conserved four-stranded  $\beta$ -sheet formed by interdigitation of the *N*- (residues 1-4) and *C*- (residues 96-99) monomer ends has been targeted<sup>2</sup>. Moreover, this 'hot spot' is responsible for 75% of the dimerization energy. We previously designed, synthesized and studied three series of molecules acting as dimerization inhibitors of HIV-1 protease: lipopeptides ( $K_{id} = 5$  nM)<sup>3</sup>, guanidinium bicyclic molecules ( $K_{id} = 150$  nM)<sup>4</sup> and molecular hairpins ( $K_{id} = 80$  nM)<sup>5</sup>. Here we describe the next generations of molecular hairpins and lipopeptides. The peptidic character of the new molecular hairpins has been decreased and even abolished by replacing amino acids with groups having similar hydrogen bonding properties. By shortening the alkyl chain and varying the peptide sequence, we obtained new lipopeptides acting *in vitro* as dimerization inhibitors at the subnanomolar level ( $K_{id} = 0,3$  nM). Their resistance to proteolysis was increased by introducing *D*-amino acids. The mechanism of inhibition was established using a combination of kinetic (Zhang-Poorman's analysis) and biophysical methods. Ultracentrifugation gave clear evidence that lipopeptides dissociate the protease dimer and sequester monomers. Molecular models of inhibitor/protease monomer complexes corroborated kinetics with an optimal occupancy of the hydrophobic enzyme cleft by the lipopeptide alkyl chain. Previous 2D[<sup>1</sup>H-<sup>13</sup>C]-HSQC NMR studies also demonstrated a non-covalent dissociative mode of interaction between the protease and an inhibitor<sup>6</sup>. Our dimerization inhibitors proved equally active *in vitro* against both wild-type and drug-resistant multi-mutated proteases in agreement with our models in which multiple mutations do not interfere with the binding of lipopeptides.

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<sup>6</sup>Frutos S, Rodriguez-Mias RA, Madurga S, Collinet B, Reboud-Ravaux M, Ludevid D, Giralt E. *Biopolymers*. 88:164-73, 2007

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