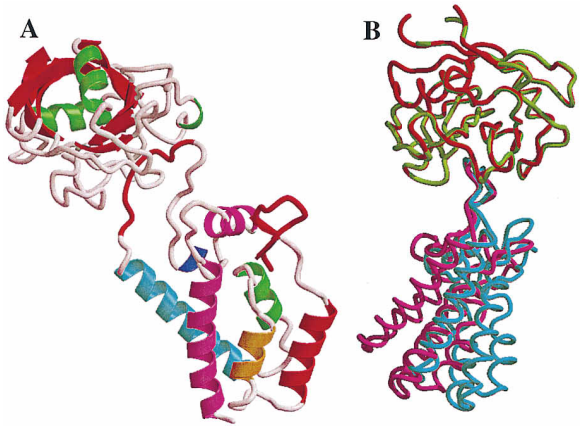

Figure 2. Interaction between CA_{151} and CypA

(A) Ribbon diagram of the CA_{151}/CypA complex: orientation of CA_{151} is similar to that of (1B), and the same color code is used. CypA β strands are shown in red and helices are in green.

(B) Tube representation of the CA_{151}/CypA complex: orientation is perpendicular to (1B). Both complexes in the asymmetric unit are shown following superposition on the two CypA molecules. Complex 1 is shown in cyan (CA_{151}-1) and green (CypA-1), and complex 2 is in magenta (CA_{151}-2) and red (CypA-2). Figures 2, 4, and 5 were prepared with MIDASPLUS (Ferrin et al., 1988).

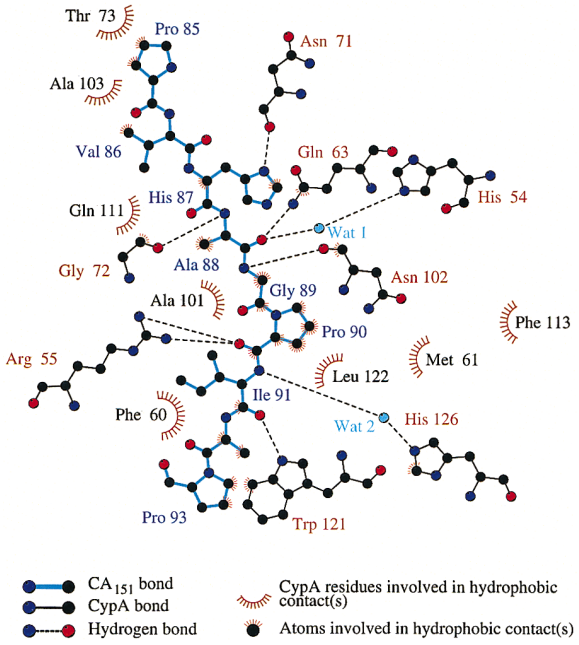

Figure 3. Molecular Recognition between CA_{151} and CypA

Schematic representation of the CA_{151} and CypA interface (Wallace et al., 1995). Hydrogen bonding interactions are shown when the acceptor-to-donor distance is 3.4 Å or less in one of the two complexes. Van der Waals interactions are defined as contacts of 4.1 Å or less in one of the two complexes.

interactions that have not been observed previously. The CA_{151} Pro-90 residue binds to CypA in the same hydrophobic pocket as seen in all known CypA–peptide structures (Kallen et al., 1991; Ke et al., 1993; Zhao and Ke, 1996a; Zhao and Ke, 1996b). However, unlike all previous structures, where bound prolines adopted the *cis* conformation, CA_{151} Pro-90 is *trans*.

Comparison of the CA_{151}/CypA structure with the structure of CypA in complex with the tetrapeptide *succinyl*-Ala-Ala-Pro-Phe-*pNA* (*pNA* = *p*-nitroanalide) (Zhao and Ke, 1996a) reveals that the CA_{151} *trans*-Pro-90 conformation has dramatic consequences for sequence-specific recognition by CypA. We restrict our detailed comparison to this tetrapeptide complex since the dipeptide complexes are less relevant because their charged termini make unusual interactions within the CypA active site (Zhao and Ke, 1996b). The CypA protein is very similar in the tetrapeptide and CA_{151} complexes, with an RMSD on all C^{α} atoms of 0.29 Å. The protein therefore appears to act as a rigid scaffold that binds the tetrapeptide proline in the *cis* conformation and the CA_{151} proline in the *trans* conformation.

As shown in Figure 4, the bound ^{87}His-Ala-Gly-Pro-Ile-Ala^{92} segment of CA_{151} and the *succinyl*-Ala-Ala-Pro-Phe-*pNA* tetrapeptide adopt very similar structures from their central proline residues back to their C-termini. However, the structures diverge significantly N-terminal to their central proline residues owing to the different conformations of the X-Pro bonds. The *trans* conformation of CA_{151} Pro-90 allows the preceding Ala-88 and Gly-89 residues to bury deep in the CypA active site and to make a series of sequence-specific interactions. This conformation is only allowed, however, by the absence of a side chain at Gly-89, since the phi/psi angles (148/155) are unfavorable for nonglycine residues. Furthermore, the hypothetical mutation of Gly-89 to Ala would place the C^{β} methyl group unacceptably close to the Arg-55 $N^{\eta 2}$ (2.5 Å) and Gln-63 $O^{\epsilon 1}$ (2.7 Å) atoms of CypA. Forced rearrangement of these CypA residues appears energetically unfavorable since the Arg-55 and Gln-63 side chains make a total of three intermolecular hydrogen bonds to the peptide and are also involved in a series of buttressing intramolecular hydrogen bonds (Figures 3 and 4). The unfavorable interaction at the Ala-89 C^{β} position could alternatively be relieved by isomerization of the Ala-Pro peptide bond, as observed in the structure of *succinyl*-Ala-Ala-Pro-Phe-*pNA* bound to CypA.

As a consequence of the CA_{151} *trans*-Pro-90 peptide bond, the preceding residues make a series of specific intermolecular interactions with CypA, including hydrogen bonds to CA_{151} His-87 $N^{\delta 1}$, Ala-88 N, Ala-88 O, and Gly-89 N and van der Waals interactions with the side chains of CA_{151} Pro-85, Val-86, His-87, and Ala-88 (see Figure 3). Apart from Gly-89 and Pro-90, all other CA_{151} side chains in contact with CypA lie along the surface of CypA rather than projecting directly towards the protein. Thus, the CA_{151}/CypA crystal structure is entirely consistent with our mutational analyses, which demonstrate that CA_{151} residues Gly-89 and Pro-90 are the primary determinants of sequence-specific CypA binding, with further contributions from Pro-85, Val-86, His-87, Ala-88, Ala-92, and Pro-93 (Yoo et al., unpublished data). Moreover, Gly-89 and Pro-90 are highly conserved in all reported HIV-1 sequences (Myers et al., 1995), and mutation of either residue to Ala inhibits CypA packaging and blocks viral infectivity in culture (Franke et al., 1994b; Braaten et al., 1996b).