Table 1. NMR/X-ray AMBER refinement data

Restraints					
Intramolecular NOEs			62 His $H^{\varepsilon 1}$ 64 - Ala H^{β} 3.8		
Intermolecular NOEs					
138 Ile $H^{\delta 2}$	Cap-1	$C1H_3$ 4.3	23 Trp $H^{\eta 2}$	Cap-1	$C1H_3$ 5.5
141 Ile $H^{\gamma 2}$	Cap-1	$C1H_3$ 4.3	27 Val $H^{\gamma 1}$	Cap-1	H6 5.5
32 Phe H^{δ}	Cap-1	H5 6.1	27 Val $H^{\gamma 1}$	Cap-1	H5 5.5
32 Phe H^{δ}	Cap-1	H3 6.1	31 Val H^{β}	Cap-1	H5 3.8
32 Phe H^{ε}	Cap-1	H5 6.1	31 Val H^{β}	Cap-1	H3 5.0
32 Phe H^{ε}	Cap-1	H3 6.1	59 Val H^{β}	Cap-1	H6 3.8
32 Phe H^{ζ}	Cap-1	H5 5.0	59 Val $H^{\gamma 2}$	Cap-1	H6 5.5
32 Phe H^{ζ}	Cap-1	H3 5.0	65 Ala H^{β}	Cap-1	H6 5.5
66 Met H^{ε}	Cap-1	H6 5.5	65 Ala H^{β}	Cap-1	H5 5.5
			65 Ala H^{β}	Cap-1	H3 5.5
Torsion angle restraints			C6-C7-N1-C8 180		
			C7-N1-C8-N2 180		
			N1-C8-N2-C9 180		
Atoms fixed to X-ray coordinates			All atoms: residues 1-25, 27-59, 63-144		
			Backbone atoms: residues 26,62		
Refined structures (20 total)[a]					
Total energy			−4928.5±1.5		
AMBER energy			−5019.0±1.5		
Restraint energy			90.5±1.4		
Distance penalty			0.000±0.000		
Torsion penalty			0.02±0.01		

[a] Energies (kcal/mol) are reported as the mean±standard deviation for the 20 refined structures.

crystallographically independent views, Phe32 has well defined density in the buried position in 14 cases, but has weak density in the other eight examples. Phe32 might be adopting a range of exposed conformations in the structures with weak density, although in no case is density for an ordered Phe32 conformation visible outside of the usual buried position.

To understand better the conformational changes that occur upon CAP-1 binding, we determined the crystal structure of a CA^N mutant (A92E) to 1.9 Å resolution in the absence of CAP-1. Ala92 resides in a flexible loop that is well removed from the CAP-1 binding site, and mutation of this residue alters HIV-1's dependence on cyclophilin A[33,34] but does not lead to global or local structural perturbations.[35] The CA^N(A92E) protein was crystallized using conditions different from those previously reported for the selenomethionine-substituted variant of this construct.[32] The eight molecules within the asymmetric unit of these crystals are very similar to those observed in previous CA^N and CA^N:cyclophilin X-ray structures, with the Phe32 side-chain buried within the folded core of the protein. Surprisingly, six of the eight molecules in the new CA^N(A92E) structure have well defined electron density for a *cis* Ala31–Phe32 peptide bond, whereas two molecules have density consistent with the previously observed *trans* peptide bonds (Figure 5). This contrasts with all previously reported CA^N structures, which have at least one well-defined *trans* Ala31–Phe32 conformation molecule and in some cases have additional molecules in the asymmetric unit that display ill-defined density but never show a well-defined *cis* Ala31–Phe32 peptide. Note that the cases of unclear densities are not easily modeled simply as a mixture of the well-defined *cis* and *trans* conformations.

The observation of a *cis* Ala31-Phe32 peptide bond in the CA^N crystal structure is surprising because *cis* peptide bonds are not typically seen before residues other than proline due to their relatively high conformational energy. The reason that the *cis* conformation is energetically accessible for Ala31–Phe32 is explained by inspection of the Ala31 main-chain. The Ala31 phi angle of *trans* conformation structures in the absence of CAP-1 is unfavorable (ranging from +43° to +57°) whereas Ala31 phi for the *cis* conformation is favorable (−60° to −75°). Thus, strain in the main-chain is manifest either as a *cis* conformation for the Ala31–Phe32 peptide or as an unfavorable phi angle for Ala31. In contrast, the CA^N structure crystallized in the presence of CAP-1 (i.e. Phe32 "out") displays both a *trans* Ala31–Phe32 peptide and a favorable Ala31 phi angle (−45°). Taken together, these observations suggest that the energetically unfavorable exposure of the Phe32 side-chain in the presence of CAP-1 is partially offset by relief of conformational strain in the main-chain. The NMR data obtained for CA^N do not exhibit signals or NOEs characteristic of a *cis* conformer, and we therefore believe that the Ala31–Phe32 bond exists predominantly as an ensemble of strained *trans* conformations under physiological conditions.

Steric strain involving non-Pro *cis* peptide bonds is rare in protein structures, and when present is usually associated with functional sites.[36,37] In this regard, Phe32 is conserved in 610 of 613 HIV-1 sequences present in the Los Alamos HIV-1 data base, and the remaining three sequences have a Phe side-chain shifted from position 32 by just one residue in the alignment.[38] Phe32 is also conserved in 63 of the 64 available HIV-2 Gag sequences (with the remaining one sequence conservatively substituted by Leu) and in 60 of the 67 available SIV sequences (substituted seven times by Trp). Taken together, these findings suggest that main-chain strain of Ala31–Phe32 may have been evolutionarily selected, perhaps to facilitate capsid assembly (see below).

Mechanism of inhibition of capsid assembly by CAP-1

CA^N functions in the assembly of both immature virions and the cone-shaped capsid that characterizes mature infectious virions. Electron microscopy (EM) studies have revealed that CA^N adopts a hexagonal lattice within the immature virion, but the precise domain orientation is not yet known.[39] The mature capsid adopts a fullerene organization in which the majority of the surface is formed by CA^N hexamers that are linked through CA^C dimers,[16,18] with five pentagonal defects distributed at the narrow end of the conical assembly and seven at the wide end.[13] Moderate resolution models for the hexagonal portion of the mature capsid have