1994) using the 1.64 Å CypA structure of Ke et al. (1993) (PDB code 2CYH) as the search model. The top solution for two CypA molecules gave a correlation coefficient of 0.43 and an R value of 45% against 7.0–3.5 Å data.

In order to build the CA_{151} structure, structure factors were calculated from the rigid body–refined CypA structure, weights were calculated with SIGMAA (Read, 1986), and phases were refined by solvent flattening and histogram shifting with dm (Cowtan, 1994). Inspection of the density allowed assignment of noncrystallographic symmetry (NCS) operators for the CA_{151} molecules and further phase refinement by 2-fold averaging. The resulting unbiased electron density map for CA_{151} was readily interpretable (Figure 7).

Rounds of refinement with X-PLOR (Brunger, 1992) were interspersed with model building using O (Jones et al., 1991). Prior to the start of atomic refinement, 10% of the data were assigned to the free R set to allow cross validation. During refinement, NCS restraints were applied to 124 residues of CypA and 70 residues of CA_{151}. NCS restraints were also applied to backbone atoms (N, C^{α}, C', O, and C^{β}) for an additional 8 and 18 residues of CypA and CA_{151}, respectively. Refinement cycles were only accepted if they gave a lower free R value. The free R value was also used to select the relative weights for X-ray and stereochemical terms and the resolution range used in refinement. All data between 6.0 and 2.36 Å were used in the refinement. The current R value is 23.8% (free R value: 30.6%). In the highest resolution shell (2.46–2.36 Å), the R value is 35.0% (free R value: 40.2%). The stereochemistry is good; RMSD (bonds) = 0.005 Å; RMSD (angles) = 1.3°; there are no φ,ψ angles in the forbidden range (Laskowski et al., 1993). A total of 107 water molecules have been located with average thermal factors (25.2 $Å^2$) that are almost identical to those of the protein (25.0 $Å^2$). Water molecules were included conservatively and were evaluated carefully with regard to appearance of the electron density, contacts with protein, and changes in the free R value. Special care was taken with CA_{151} Pro-90 because the equivalent proline is *cis* in all previously reported CypA–peptide complexes. Refinement calculations and electron density clearly indicate the *trans* conformation.

Regions that lack well-defined density have been given an occupancy of zero for crystallographic refinement and for R value calculations. Residues with zero occupancy are: CypA-1; 80, 81, and 165, CypA-2; 80 and 81, CA_{151}-1; 6–8, CA_{151}-2; and 1–11, 31, 60–63, and 95–96. The following residues were given zero occupancy beyond their C^{β} atoms: CypA-1; 15, 91, and 144, CypA-2; 1, 15, 69, 144, and 151, CA_{151}-1; 4, 18, 25, 30, 35, 63, 95, 112, 121, and 128, CA_{151}-2; and 18, 21, 28–30, 35, 45, 50, 67, 70–71, 112, 128, and 140. The following residues were completely omitted from the model: CypA-1; 1–2,165: CA_{151}-1; 146–151: and CA_{151}-2; 146–151.

The N-terminal hairpin of CA_{151} is well defined for CA_{151}-1, except for residues 6–8. However, in CA_{151}-2, none of the residues preceding His-12 have well-defined density. Some density was evident for residues 1–3 and 10–11, although model building into this density relied heavily on the conformation seen in CA_{151}-1.

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Correspondence and requests for materials should be addressed to W. I. S. (sundquist@medschool.med.utah.edu) or C. P. H. (chris@msscc.med.utah.edu). Coordinates and diffraction data will be deposited with the Brookhaven Protein Database.

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References

Aberham, C., Weber, S., and Phares, W. (1996). Spontaneous mutations in the human immunodeficiency virus type 1 *gag* gene that affect viral replication in the presence of cyclopsorins. J. Virol. *70*, 3536–3544.

Anderson, S.K., Gallinger, S., Roder, J., Frey, J., Young, H.A., and Ortaldo, J.R. (1993). A cyclophilin-related protein involved in the function of natural killer cells. Proc. Natl. Acad. Sci. USA *90*, 542–546.

Bacon, D.J., and Anderson, W.F. (1988). A fast algorithm for rendering space-filling molecule pictures. J. Molec. Graphics *6*, 219–220.

Baker, E.K., Colley, N.J., and Zuker, C.S. (1994). The cyclophilin homolog NinaA functions as a chaparone, forming a stable complex in vivo with its protein target rhodopsin. EMBO J. *13*, 4886–4895.

Billich, A., Hammerschmid, F., Peichl, P., Wenger, R., Zenke, G., Quesniaux, V., and Rosenwirth, B. (1995). Mode of action of SDZ NIM 811, a nonimmunosuppressive cyclosporin A analog with activity against human immunodeficiency virus (HIV) type 1: Interference with HIV protein-cyclophilin A interactions. J. Virol. *69*, 2451–2461.

Braaten, D., Aberham, C., Franke, E.K., Yin, L., Phares, W., and Luban, J. (1996a). Cyclosporine A-resistant human immunodeficiency virus type 1 mutants demonstrate that gag encodes the functional target of cyclophilin A. J. Virol. *70*, 5170–5176.

Braaten, D., Franke, E.K., and Luban, J. (1996b). Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. J. Virol. *70*, 3551–3560.

Braaten, D., Franke, E.K., and Luban, J. (1996c). Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus $SIV_{CPZ}GAB$ but not group O HIV-1 or other primate immunodeficiency viruses. J. Virol. *70*, 4220–4227.

Bram, R.J., and Crabtree, G.R. (1994). Calcium signalling in T cells stimulated by a cyclophilin B-binding protein. Nature *371*, 355–358.

Brunger, A.T. (1992). X-PLOR, a system for X-ray crystallography and NMR. Vol. Version 3.1, Yale University. (New Haven, Connecticut: Yale University Press).

Colgan, J., Yuan, H.E.H., Franke, E.K., and Luban, J. (1996). Binding of the human immunodeficiency virus type 1 gag polyprotein to cyclophilin A is mediated by the central region of capsid and requires gag dimerization. J. Virol. *70*, 4299–4310.

Colley, N.J., Baker, E.K., Stamnes, M.A., and Zuker, C.S. (1991). The cyclophilin homolog NinaA is required in the secretory pathway. Cell *67*, 255–263.

Cowtan, K.D. (1994). 'dm': an automated procedure for phase improvement by density modification. Joint CCP4 and ESF-EACBM newsletter on protein crystallography *31*, 34–38.

Dorfman, T., Bukovsky, A., Öhagen, Å., Höglund, S., and Göttlinger, H.G. (1994). Functional domains of the capsid protein human immunodeficiency virus type 1. J. Virol. *68*, 8180–8187.

Dorfman, T., Luban, J., Goff, S.P., Haseltine, W.A., and Göttlinger, H.G. (1993). Mapping of functionally important residues of a cysteine-histidine box in the human immunodeficiency virus type 1 nucleocapsid protein. J. Virol. *67*, 6159–6169.

Ferrin, T.E., Huang, C.C., Jarvis, L.E., and Langridge, R. (1988). The MIDAS display system. J. Mol. Graphics *6*, 13–27.

Franke, E.K., Yuan, H.E.H., Bossolt, K.L., Goff, S.P., and Luban, J. (1994a). Specificity and sequence requirements for interactions between various retroviral gag proteins. J. Virol. *68*, 5300–5305.

Franke, E.K., Yuan, H.E.H, and Luban, J (1994b). Specific incorporation of cyclophilin A into HIV-1 virions. Nature *372*, 359–362.

Gelderblom, H.R., Bauer, P.G., Özel, M., Höglund, S., Niedrig, M., Renz, H., Morath, B., Lundquist, P., Nilsson, Å., Mattow, J., et al. (1992). Morphogenesis and morphology of human immunodeficiency virus. In Membrane Interactions of HIV, R.C. Aloia, and C.C. Curtain, eds. (New York: Wiley-Liss, Inc.), pp. 33–54.

Gitti, R.K., Lee, B.M., Walker, J., Summers, M.F., Yoo, S., and Sundquist, W.I. (1996). Structure of the amino-terminal core domain of the HIV-1 capsid protein. Science *273*, 231–235.

Harrison, R.K., and Stein, R.L. (1990a). Mechanistic studies of peptidyl prolyl cis-trans isomerase: evidence for catalysis by distortion. Biochemistry *29*, 1684–1689.