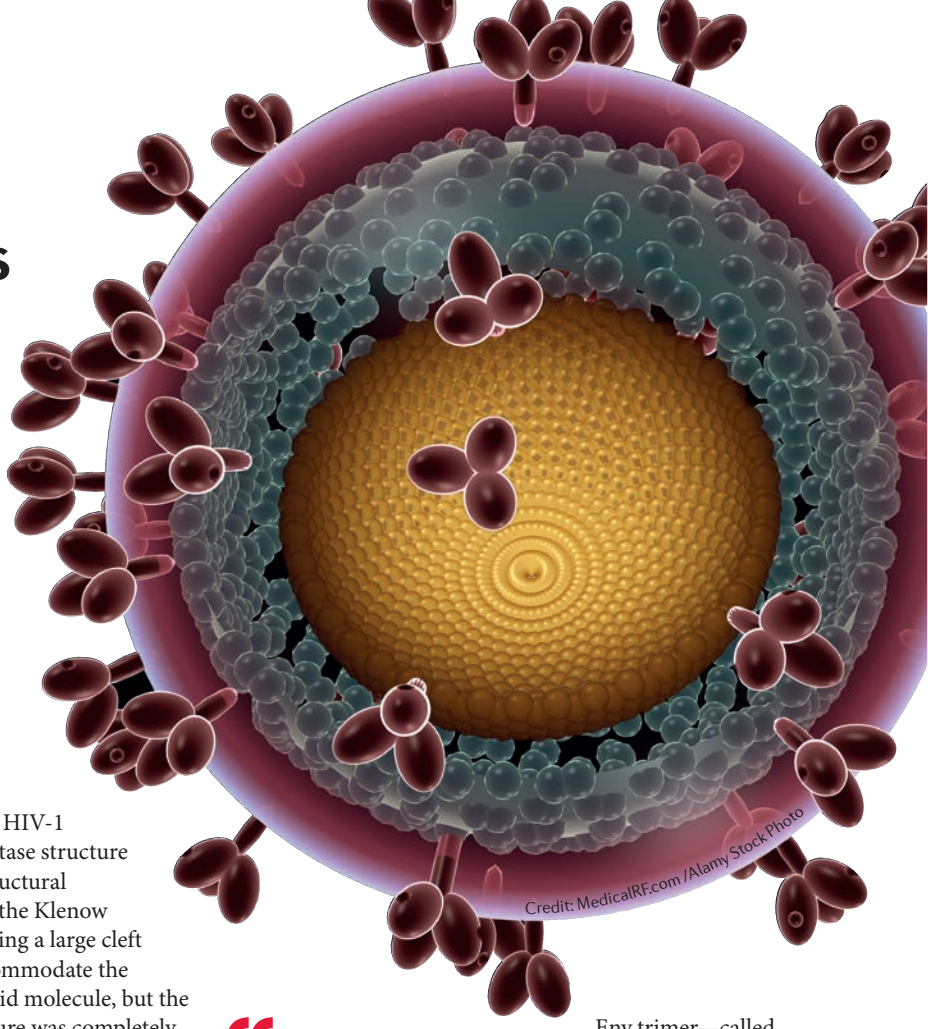
MILESTONE 8

Structural insights into HIV proteins



Soon after the discovery of HIV-1, intense efforts focused on developing drugs to tackle the virus. Early targets were the HIV-1 protease and reverse transcriptase, and drug development was greatly assisted by breakthroughs in solving the structures of these viral proteins.

The first HIV-1 protein to yield a high-resolution structure was the HIV-1 protease. Scientists at Merck Sharp and Dohme Research Laboratories published the first structure in 1989, using recombinant protease expressed in bacteria, which revealed essential features of the catalytic apparatus. Soon after, using a chemical synthesis approach to obtain enough protein for crystallization and some modeling based on the Rous sarcoma virus protease structure, scientists at NCI-Frederick obtained a 2.8-Ångstrom structure of the HIV-1 protease in which all 99 amino acids could be located. Shortly afterward, the first co-crystal structure of its complex with an inhibitor was determined, paving the way for rapid drug development and approval of the first protease inhibitor for HIV-1 therapy six years later in 1995 (MILESTONE 14).

The first drug to treat HIV, however, was approved in 1987, and it targeted the viral reverse transcriptase. Although this drug, azidothymidine (AZT), was rather poor, many of the drugs used today to treat HIV infection target this enzyme: nucleoside reverse-transcriptase inhibitors (NRTIs) become incorporated into viral DNA by the action of reverse transcriptase and block viral RNA synthesis, whereas non-nucleoside reverse-transcriptase inhibitors (NNRTIs) inhibit the enzyme by direct binding.

In 1992, Thomas Steitz and colleagues provided the first high-resolution structural glimpse of how an NNRTI, nevirapine, interacted with the HIV-1 reverse transcriptase enzyme. Before this time, the mechanism by which nevirapine worked was unknown, and furthermore, the only polymerase for which there was structural information was the Klenow fragment from *Escherichia coli* polymerase. The HIV-1 reverse transcriptase structure showed some structural similarities with the Klenow fragment, including a large cleft sufficient to accommodate the RNA–DNA hybrid molecule, but the rest of the structure was completely different. The structure also revealed where nevirapine bound, suggesting potential mechanisms by which the drug inhibits reverse transcriptase as well as revealing the location of known resistance-conferring mutations. This paper and related structural work from Edward Arnold and colleagues published around the same time, as well as structures that soon followed, set the stage for the design of more effective reverse transcriptase inhibitors—drugs that remain mainstays of treatment today.

The HIV-1 envelope protein, Env, which mediates fusion of the virus with the cell membrane, has also been a focus of intense interest—mainly for vaccine design (MILESTONE 9). But this conformationally plastic protein proved difficult to study. Crystal structures of the postfusion conformation of gp41 were solved by the groups of Peter Kim and Don Wiley in 1997 and that of the CD4-bound conformation of gp120 was solved by the group of Wayne Hendrickson in 1998. In 2002, John Moore and colleagues reported a stabilized version of the Env trimer—called SOSIP—in which the gp120 and gp41 subunits were held together with an engineered disulfide bond. But it took another decade to obtain high-resolution structures of this SOSIP trimer—eventually determined simultaneously through crystallography and cryo-EM, by the groups of Andrew Ward and Ian Wilson. The information obtained from these structures and others that have followed is hoped to aid the design of an effective vaccine.

> “drug development was greatly assisted by breakthroughs in solving the structures of these viral proteins”

Clare Thomas, *Nature*

ORIGINAL ARTICLES Navia, M. A. et al. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. *Nature* **337**, 615–620 (1989) | Wlodawer, A. et al. Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease. *Science* **245**, 616–621 (1989) | Swain, A. L. et al. X-ray crystallographic structure of a complex between a synthetic protease of human immunodeficiency virus 1 and a substrate-based hydroxyethyamine inhibitor. *Proc. Natl. Acad. Sci. USA* **87**, 8805–8809 (1990) | Arnold, E. et al. Structure of HIV-1 reverse transcriptase/DNA complex at 7 Å resolution showing active site locations. *Nature* **357**, 85–89 (1992) | Kohlstaedt, L. A., Wang, J., Friedman, J. M., Rice, P. A. & Steitz, T. A. Crystal structure at 3.5 Å resolution of HIV-1 reverse transc riptase complexed with an inhibitor. *Science* **256**, 1783–1790 (1992) | Chan, D. C., Fass, D., Berger, J. M. & Kim, P. S. Core structure of gp41 from the HIV envelope glycoprotein. *Cell* **89**, 263–273 (1997) | Weissenhorn, W., Dessen, A., Harrison, S. C., Skehel, J. J. & Wiley, D. C. Atomic structure of the ectodomain from HIV-1 gp41. *Nature* **387**, 426–430 (1997) | Kwong, P. D. et al. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **393**, 648–659 (1998) | Sanders, R. W. et al. Stabilization of the soluble, cleaved trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. *J. Virol.* **76**, 8875–8889 (2002) | Lyumkis, D. et al. Cryo-EM structure of a fully glycosylated soluble cleaved HIV-1 envelope trimer. *Science* **342**, 1484–1490 (2013) | Julien, J. et al. Crystal structure of a soluble cleaved HIV-1 envelope trimer. *Science* **342**, 1477–1483 (2013)