Mechanism of allosteric activation of SAMHD1 by dGTP

Xiaoyun Ji1,6, Ying Wu2,3,6, Junpeng Yan4,6, Jennifer Mehrens2,3, Haitao Yang1,5, Maria DeLucia2,3, Caili Hao4, Angela M Gronenborn2,3, Jacek Skowronski3,4, Jinwoo Ahn2,3 & Yong Xiong1

SAMHD1, a dNTP triphosphohydrolase (dNTPase), has a key role in human innate immunity. It inhibits infection of blood cells by retroviruses, including HIV, and prevents the development of the autoimmune Aicardi–Goutières syndrome (AGS). The inactive apo-SAMHD1 interconverts between monomers and dimers, and in the presence of dGTP the protein assembles into catalytically active tetramers. Here, we present the crystal structure of the human tetrameric SAMHD1–dGTP complex. The structure reveals an elegant allosteric mechanism of activation through dGTP-induced tetramerization of two inactive dimers. Binding of dGTP to four allosteric sites promotes tetramerization and induces a conformational change in the substrate-binding pocket to yield the catalytically active enzyme. Structure-based biochemical and cell-based biological assays confirmed the proposed mechanism. The SAMHD1 tetramer structure provides the basis for a mechanistic understanding of its function in HIV restriction and the pathogenesis of AGS.

The sterile alpha motif and HD domain–containing protein 1 (SAMHD1) dNTPase has dual roles in human innate immunity. It restricts HIV-1 infection in immune cells of myeloid lineage and in quiescent CD4-positive T lymphocytes1–5. In these nondividing cells, SAMHD1 reduces cellular dNTP levels to concentrations below the threshold required for reverse transcription of the viral RNA genome into DNA6–8. Furthermore, mutations in SAMHD1 are associated with an autoimmune condition, AGS9,10, whose clinical manifestations resemble congenital viral infection11,12. AGS-associated SAMHD1 mutations appear to disrupt the dNTPase activity of SAMHD1. Thus, SAMHD1’s ability to negatively regulate cellular dNTP levels is essential for its roles in innate immunity13,14.

The dNTPase activity of SAMHD1 resides in its histidine-aspartate (HD) domain, with the N-terminal sterile alpha motif (SAM) domain involved in other activities13–17. A recent crystal structure of a dimeric SAMHD1 catalytic-core fragment (SAMHD1c1, residues 120–626) suggested an allosteric, dGTP-stimulated mechanism for the promotion of the dNTPase activity of dimeric SAMHD1 (ref. 14). However, the SAMHD1c1 structure did not contain substrate or the dGTP cofactor, thus providing limited insight into the mechanism of SAMHD1 activation. Recent biochemical and functional studies revealed that SAMHD1 interconverts between an inactive monomeric or dimeric form and a dGTP-induced tetrameric form that is the active dNTPase that restricts HIV15,18.

To elucidate the mechanism of dGTP-induced oligomerization and allosteric activation of SAMHD1, we determined the crystal structure of the tetrameric SAMHD1–dGTP complex. The structure revealed two dGTP molecules in each of the four allosteric sites of the SAMHD1 tetramer. Comparison of the apo-SAMHD1 dimer with the SAMHD1–dGTP tetramer structures showed large, allosteric dGTP-induced conformational changes at the tetramer interfaces and the catalytic sites nearby, thus enabling the binding of the dNTP substrates. We validated the importance of the residues involved in these changes by using biochemical and cell-biological assays. Together, our results provide mechanistic insights into dGTP-induced allosteric regulation of the catalytic function of SAMHD1.

RESULTS

To investigate the allosteric activation of SAMHD1 by dGTP, we determined the crystal structure of the human SAMHD1 catalytic core (SAMHD1c2, residues 113–626) bound to dGTP (Fig. 1 and Table 1). SAMHD1c2 undergoes dGTP-dependent tetramerization and activation similarly to the full-length wild-type protein15. To prevent substrate hydrolysis, we used the substrate analog α-thio-dGTP (dGTPαS) to trap the complex in the substrate-bound conformation and determined the dGTPαS–SAMHD1c2 structure at 2.9-Å resolution. In addition, we solved the structure of a catalytically inactive mutant, SAMHD1c2-RN (H206R D207N), with bound dGTP at 1.8-Å resolution. The two structures are virtually identical (r.m.s. deviation 0.53 Å), except that the mutant complex does not contain the catalytic metal ion, owing to substitution of the conserved metal-coordinating residues H206 and D207 by arginine and asparagine, respectively. For clarity, we refer to the structures as the dGTP–SAMHD1c2 complex when describing identical features in both structures. The overall structure comprises a well-defined catalytic core, with no density observed for only a short loop region (residues 278–283) and the C terminus (residues 600–626).

1Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, USA. 2Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA. 3Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA. 4Department of Molecular Biology and Microbiology, Case Western Reserve School of Medicine, Cleveland, Ohio, USA. 5Present address: School of Life Sciences, Tianjin University, Tianjin, China. 6These authors contributed equally to this work. Correspondence should be addressed to J.A. (jahn@structbio.pitt.edu) or Y.X. (yong.xiong@yale.edu).

Received 6 June; accepted 10 September; published online 20 October 2013; doi:10.1038/nsmb.2692
The structure of the dGTP-induced SAMHD1 tetramer

One SAMHD1c2 tetramer of 222 point-group symmetry (Fig. 1a,b; subunits labeled A, B, C and D) is in the asymmetric unit of the crystal. The AD and BC dimer structures are similar to the previously reported dimer structure of SAMHD1c1, burying 1,750 Å² surface area per subunit at the dimer interface. These AD and BC interfaces are referred to as the dimer-1 interfaces in the following discussion. Two dimers associate further and form a very tight tetramer, thus resulting in a buried dimer-dimer interface of 4,920 Å². The tetramerization is mediated mainly by the newly formed interfaces between A and C and between B and D. We refer to the AC and BD interfaces as the dimer-2 or tetramer interfaces. The subunits in the tetramer exhibit a virtually identical conformation, which is notably different from that in the dimeric SAMHD1c1 structure (Fig. 1c and Supplementary Fig. 1a). In particular, we observed a large conformational change at the new dimer-2 or tetramer interface (Fig. 1d). Tetramerization causes ordering of over 40 residues (residues 506–515, 531–547 and 584–599, with each end labeled) in SAMHD1c1 (Supplementary Fig. 2 and Supplementary Fig. 1a). These residues are critical for the formation of the SAMHD1 tetramer (discussed below).

The most striking feature in the SAMHD1c2 tetramer structure is the presence of 12 dGTP molecules. There are two dGTPs bound to each of the four allosteric sites and one dGTP bound to each of the four catalytic sites (Fig. 1b). Each allosteric dGTP-binding site is located at an interface of three subunits, thus highlighting the importance of dGTP for mediating the tetramer structure (Fig. 1a). This structural result agrees well with our solution biochemical data of dGTP-induced tetramerization.15 Notably, residues 113–119 form a critical part of the allosteric dGTP-binding pocket (Figs. 1c and 2a), thus explaining why SAMHD1c1, which lacks these residues, crystallized only as a dimer.14

dGTP–Mg²⁺–dGTP binding at the allosteric site of SAMHD1

The allosteric dGTPs interact with an extensive network of hydrogen-bonding residues and exhibit excellent shape and size complementarity with the binding pocket at a three-subunit interface (Fig. 2a). Because all four allosteric dGTP-binding sites are identical, we selected the ACD interface for discussion of interactions. One of the two dGTPs (designated dGTP-1) interacts with residues D137, Q142 and R145 of subunit D, forming five hydrogen-bonding interactions with the Watson-Crick and the Hoogsteen (N7) sites of the G base. The second allosteric dGTP (designated as dGTP-2) interacts at the Watson-Crick base sites with residues N358 and D330 of subunit C. In addition, numerous residues from subunits A (V156, H376, K377, R451 and K455), C (R333, R352, K354 and K523) and D (K116 and N119) provide additional hydrogen-bonding and stacking interactions with the base, sugar and triphosphate moieties of the two allosteric dGTPs (Fig. 2b and Supplementary Fig. 2). Any other base in this pocket would result in fewer hydrogen bonds or in steric hindrance in the binding site. This provides a clear structural rationale for the inability of other dNTPs to activate SAMHD1 (ref. 14). The size of the dGTP-binding pocket provides a snug fit to the deoxyribose ring, leaving little room for the 2’-hydroxyl group of a ribose of GTP (Supplementary Fig. 3a).

The two allosteric dGTPs are juxtaposed with a Mg²⁺ ion bridging their phosphate groups in a dGTP–Mg²⁺–dGTP configuration (Fig. 2a and Supplementary Fig. 3b), and four such dGTP pairs at the four allosteric sites interlock the subunits in the SAMHD1 tetramer.
of interaction resides in the center of the tetramer with a loop-helix region (residues 325–333) engaging in direct protein-protein contacts as well as contacts to dGTP-2 (Fig. 2f). This further demonstrates the critical role that dGTP has in SAMHD1 tetramerization.

Phosphorylation of SAMHD1 residue T592 has been reported recently as a key regulator of its antiviral function. The phosphorylation is catalyzed by the cell-cycle regulator cyclin A2–CDK1 upon recognition of the target motif (592-TPQK-595) on SAMHD1 (refs. 22–24). Our structure shows that T592 resides in the newly ordered C-terminal region whose structure is stabilized by tetramerization (Fig. 2d). The 592-TPQK-595 motif is close to the tetramer interface and is solvent exposed (Supplementary Fig. 4). Modeling of a phosphate group onto T592 suggests that phosphorylation does not affect tetramerization or the folding of the protein. This is consistent with the finding that phosphorylation of T592 does not change the dNTPase activity of SAMHD1 (refs. 22,23).

Tetramer-disrupting mutations abolish SAMHD1 function

We carried out mutagenesis studies to verify that the contacts with the allosteric dGTPs and the associated tetramerization are important. Residue changes such as R333A, D330A N358A and R352A H376A inactivating residues engaged in the interactions along the tetramer interface and is solvent exposed (Supplementary Fig. 4). Modeling of a phosphate group onto T592 suggests that phosphorylation does not affect tetramerization or the folding of the protein. This is consistent with the finding that phosphorylation of T592 does not change the dNTPase activity of SAMHD1 (refs. 22,23).

Tetramerization of SAMHD1 activates the enzyme

In addition to the dGTP–Mg$^{2+}$–dGTP bound at the allosteric sites, one dGTP molecule is bound in the catalytic site of each SAMHD1c2 subunit (Fig. 4a). The substrate-binding pocket is located near the dimer-2 interface, with the interface α-helix (residues 352–373) (Fig. 2e) lining one side of the pocket (Fig. 4a,b). The other side of the binding pocket is close to the dimer-1 interface observed previously and contains the canonical histidine and aspartate (HD) residues required for catalysis (Fig. 4c). A catalytic metal ion is coordinated by the side chains of residues H167, H206, D207 and D311, and the oxygen of the δ-phosphate group of the substrate (Supplementary Fig. 5). Comparison of the current dGTP–SAMHD1c2 structure with that of a homologous protein, EF143 from Enterococcus faecalis (PDB 3IRH)19, suggests that H210, H233 and D218 are the catalytic residues (Supplementary Fig. 5). The extensive network of interactions within the snug binding pocket orients dGTP for catalysis (Supplementary Fig. 5). NTPs are excluded as substrates because the 2′-hydroxyl group of a ribose would clash with residues L150 and Y374 in the pocket. Notably, the extra space and lack of specific interactions at the base edge result in the ability of SAMHD1 to accommodate all four dNTPs as substrates (Supplementary Fig. 5).

The dGTP-induced tetramerization and conformational change alter the size and shape of the substrate-binding pocket in the inactive dimer to enable dNTP binding by SAMHD1 (Fig. 4c). Little change appears at the dimer-1 interface, while large structural rearrangements occur at the dimer-2 interface in SAMHD1c2 compared to SAMHD1c1 (Figs. 4a and 1d). A more open pocket in the inactive dimer closes up upon tetramerization, enclosing the dNTP substrate (Fig. 4c). Specifically, the α-helix sandwiched between the catalytic...
Figure 2 dGTP–Mg\(^{2+}\)–dGTP binding at the allosteric site induces tetramerization of SAMHD1. (a) Left, the SAMHD1c2 tetramer in surface representation, in the same color scheme as in Figure 1. Right, the allosteric dGTP-binding site between subunits A, C and D, with the D subunit shown in semitransparent ribbon representation to clearly illustrate the buried dGTP–Mg\(^{2+}\)–dGTP (yellow and orange sticks). Yellow sphere, magnesium ion. Black ribbon, residues 113–119 of subunit D. (b) Interactions between the two dGTP molecules and their surrounding residues. Residues that are not observed in the previous SAMHD1c1 dimer structure are highlighted in magenta. The three layers of interactions at the AC interface are boxed. The black oval and line indicate a two-fold symmetry axis. (d) Interactions between the two dGTP molecules and their surrounding residues. (d) Details of the interactions in the three different layers: first (d), second (e) and third (f). Several key residues are shown in stick representation. The black oval in the center represents a two-fold axis perpendicular to the given view.

Figure 3 Mutagenesis of SAMHD1 residues involved in dGTP binding and tetramerization. (a) Quantification of SAMHD1 tetramer by analytical size-exclusion chromatography\(^{15}\), expressed as a percentage of total protein. The experiments were performed in duplicate, and average values are shown. Mutated amino acid positions are indicated at top. WT, wild type. (b) In vitro dNTPase assay of wild-type and mutant SAMHD1 proteins. The nucleoside dNTPase activities (d) of wild-type SAMHD1 and mutants for the allosteric dGTP-binding site and the tetramer interface. Quantified luciferase activity\(^{15}\) in U937 cell extracts is shown in arbitrary units. dATP pools in U937 cells are presented in pmol/10\(^6\) cells. Wild-type and mutant SAMHD1 detected by immunoblotting for the N-terminal epitope tag and α-tubulin used as a loading control are shown. U937 cells transduced with an empty MSCV (puro) vector (M) were used as a negative control. Luciferase reporter activity or dNTP levels in cells expressing wild-type or mutant SAMHD1 proteins are plotted as a function of protein expression levels, normalized to the highest expression level of wild-type SAMHD1. The values of luciferase activity and dATP amounts shown are averages of three replicates. Error bars, s.d. Similar results were obtained in two independent experiments. Original images of blots used in this study can be found in Supplementary Figure 6.
transition and associated conformational changes in each cycle. This is supported by our previous biochemical data showing that SAMHD1 tetramers are stable in the presence of dGTP15.

The antiviral activity of SAMHD1 is downregulated by phosphorylation at residue T592 by cyclin A2–CDK1 kinase, thus resulting in inhibition of HIV infection only in nondividing cells such as quiescent CD4-positive cells, macrophages and dendritic cells1–4. Our structural result shows that the 592-TPQK-595 motif is solvent exposed and surface representation, respectively. The dGTP substrate is depicted in green and the metal ion as a green sphere. Missing regions in SAMHD1c1 are highlighted in magenta in the SAMHD1c2 structure. (c) Comparison of the substrate-binding pockets (surface representation) in the two structures. The catalytic residues are shown on the left, and key contact residues are shown on the right. The dGTP (gray) in the dimer is modeled on the basis of the position in the tetramer. (d) dATPase assay for WT SAMHD1 and substrate binding-site mutants. Each experiment was performed in triplicate. Error bars, s.d.

DISCUSSION

SAMHD1 has been identified as an HIV-restriction factor in nondividing cells, and the allosteric dGTP-mediated dNTPase activity is key to its antiviral function. However, the mechanism of allosteric activation of SAMHD1 was unknown. In particular, how allosteric dGTP induces the tetramerization of SAMHD1 and subsequently enables substrate-dNTP binding and catalysis remained mysterious. The work presented here establishes the structural and biochemical basis for the catalytic mechanism of SAMHD1 (Fig. 4d), presumably because they are important for binding and orienting the dGTP substrate for catalysis.

The loss-of-function mutations of SAMHD1 also lead to the autoinflammatory syndrome AGS16. Previous studies revealed that some of the AGS-linked SAMHD1 mutations disrupt the catalytic site (R143H/C and G209S). Our current structure now illustrates how other mutations such as those residing at the allosteric dGTP-binding sites (R145Q, H123P, A120–123 and M385V) or at the tetramer interfaces (L369S) interfered with SAMHD1 function. Overall, our results provide compelling evidence for the biological relevance of the SAMHD1 tetramer and offer a biochemical and structural basis for understanding SAMHD1 functions in the pathogenesis of both HIV infection and AGS.

Our structural results not only provide mechanistic insights into the regulation of SAMHD1 but also inform on the broad class of HD domain–containing allosteric phosphohydrolases that oligomerize...
and are activated upon NTP or dNTP binding\textsuperscript{19,20,27–29}. For these HD enzymes, it has been an open question how ligand binding at an allosteric site induces their activation. Our current structure suggests an elegant general mechanism, namely the creation of an active enzyme by dNTP or NTP-induced oligomerization and reshaping of the substrate-binding pocket adjacent to the newly formed molecular interface (Figs. 4c and 5).

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** Coordinates and structural factors have been deposited in the Protein Data Bank under accession codes 4BZC for the wild type and 4BZB for the RN mutant.

**Note:** Any Supplementary Information and Source Data files are available in the online version of the paper.

**ACKNOWLEDGMENTS**

We thank X. Jia, E. Weber, J. Fribourgh, H. Nguyen and B. Summers for assistance and discussion and Y. Zuo, Y. Li and C. Wang for technical assistance. We also thank the staff at the Advanced Photon Source beamline 24-ID and the National Synchrotron Light Source beamline X25. We thank N. Landau (New York University School of Medicine), T. Hope (Northwestern University) and D. McDonald (Case Western Reserve University) for supplying reagents. This work was supported in part by funds from the University of Pittsburgh School of Medicine (J.A.) and by US National Institutes of Health grants AI097064 (Y.X.), AI100673 (J.S.) and P50GM082251 (A.M.G. and J.A.).

**AUTHOR CONTRIBUTIONS**

X.J. performed protein crystallization, structure determination and structure analysis; X.J. and H.Y. collected the diffraction data; Y.W., J.M., M.D. and J.A. performed the biochemical mutation experiments and data analysis in vitro; J.Y. and C.H. performed the cell-based experiments; J.A. provided experimental materials; X.J., Y.W., J.Y., H.Y., A.M.G., J.S., J.A. and Y.X. analyzed the data; X.J., Y.W., J.Y., J.A. and Y.X. designed the experiments; and X.J., H.Y., A.M.G., J.S., J.A. and Y.X. wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Descours, B. et al. SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4+ T-cells. *Retrovirology* 9, 87 (2012).
2. Baldauf, H.M. et al. SAMHD1 restricts HIV-1 infection in resting CD4+ T cells. *Nat. Med.* 18, 1682–1687 (2012).
3. Laguette, N. et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 474, 654–661 (2011).
4. Hrecza, K. et al. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* 474, 654–661 (2011).
5. Berger, A. et al. SAMHD1-deficient CD4+ cells from individuals with Aicardi-Goutieres syndrome are highly susceptible to HIV-1 infection. *PLoS Pathog.* 7, e1002425 (2011).
6. St Gelais, C. et al. SAMHD1 restricts HIV-1 infection in dendritic cells (DCs) by dNTP depletion, but its expression in DCs and primary CD4+ T-lymphocytes cannot be upregulated by interferons. *Retrovirology* 9, 105 (2012).
7. Lahuassa, H. et al. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nat. Immunol.* 13, 223–228 (2012).
8. Kim, B., Nguyen, L.A., Daddacha, W. & Hollenbaugh, J.A. Tight interplay among SAMHD1 protein level, cellular dNTP levels, and HIV-1 proviral DNA synthesis kinetics in human primary monocyte-derived macrophages. *J. Biol. Chem.* 287, 21570–21574 (2012).
9. Xin, B. et al. Homozygous mutation in SAMHD1 gene causes cerebral vasculopathy and early onset stroke. *Proc. Natl. Acad. Sci. USA* 108, 5372–5377 (2011).
10. Rice, G.I. et al. Mutations involved in Aicardi-Goutieres syndrome implicate SAMHD1 as regulator of the innate immune response. *Nat. Genet.* 41, 829–832 (2009).
11. Crow, Y.J. & Rehinken, J. Aicardi-Goutieres syndrome and related phenotypes: linking nucleic acid metabolism with autoimmunity. *Hum. Mol. Genet.* 18, R130–R136 (2009).
12. Jepps, H., Seal, S., Hattingh, L. & Crow, Y.J. The neonatal form of Aicardi-Goutières syndrome masquerading as congenital infection. *Early Hum. Dev.* 84, 783–785 (2008).
13. Powell, R.D., Holland, P.J., Hollis, T. & Perrino, F.W. Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. *J. Biol. Chem.* 286, 43596–43600 (2011).
14. Goldstone, D.C. et al. hnRN1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature* 480, 379–382 (2011).
15. Yan, J. et al. Tetramerization of SAMHD1 is required for biological activity and inhibition of HIV infection. *J. Biol. Chem.* 288, 10406–10417 (2013).
16. White, T.E. et al. Contribution of SAM and HD domains to retroviral restriction mediated by human SAMHD1. *Virology* 436, 81–90 (2013).
17. Brandanz-Nuñez, A. et al. Role of SAM HD1 nuclear localization in restriction of HIV-1 and SIgM. *Retrovirology* 9, 49 (2012).
18. DeLucia, M., Mehrisz, J., Wu, Y. & Ahn, J. HIV-2 and SIgM accessory virulence factor Vpx down-regulates SAMHD1 catalysis prior to proteasome-dependent degradation. *J. Biol. Chem.* 288, 19116–19126 (2013).
19. Vorontsov, I.I. et al. Characterization of the deoxynucleotide triphosphate triphosphohydrolase (dNTPase) activity of the EF1143 protein from Enterococcus faecalis and crystal structure of the activator-substrate complex. *J. Biol. Chem.* 286, 33158–33166 (2011).
20. Oganesyan, V., Adams, P.D., Jancarik, J., Kim, R. & Kim, S.H. Structure of O67745_AQUAE, a hypothetical protein from *E. faecalis* and crystal structure of the activator-substrate complex. *J. Biol. Chem.* 286, 33158–33166 (2011).
21. Aravind, L. & Koonin, E.V. The HD domain defines a new superfamily of metal-dependent phosphohydrolases. *Trends Biochem. Sci.* 23, 469–472 (1998).
22. White, T.E. et al. The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation. *Cell Host Microbe* 13, 441–451 (2013).
23. Ondo, A., Deshpande, A., Malhotra, N. & Bhat, K. Activation of SIV in dendritic cells by Vpx and inhibition of SIV replication in infected cells. *FASEB J.* 28, 479–490 (2014).
24. Errico, A., Deshmukh, K., Tanaka, Y., Pozniakovsky, A. & Hunt, T. Identification of substrates for cyclin dependent kinases. *FASEB J.* 28, 479–490 (2014).
25. Beloglova, N. et al. Nucleasic activity of the human SAMHD1 protein implicated in the Aicardi-Goutieres Syndrome and HIV-1 restriction. *J. Biol. Chem.* 288, 8101–8110 (2013).
26. Goncalves, A. et al. SAMHD1 is a nucleic-acid binding protein that is mislocalized due to Aicardi-Goutieres syndrome-associated mutations. *Hum. Mutat.* 33, 1116–1122 (2012).
27. Zimmerman, M.D., Proudfoot, M., Yakunin, A. & Minor, W. Structural insight into the mechanism of substrate specificity and catalytic activity of an HD-domain phosphohydrolase: the S‘-deoxynucleoside-5‘-deoxyribonucleotide YbdR from *Escherichia coli*. *J. Mol. Biol.* 378, 215–226 (2008).
28. Kondo, N. et al. Structure of dNTP-inducible dNTP triphosphohydrolase: insight into broad specificity for dNTPs and triphosphohydrolase-type hydrolysis. *Acta Crystallogr. D Biol. Crystallogr.* 63, 230–239 (2007).
29. Kondo, N., Kuramitsu, S. & Masui, R. Biochemical characterization of T1383 from *Thermus thermophilus* identifies a novel dNTP triphosphohydrolase activity stimulated by dNTP and dTTP. *J. Biochem.* 136, 221–231 (2004).
30. Stuart, D.I., Levine, M., Murhead, H. & Stammers, D.K. Crystal structure of cat muscle pyruvate kinase at a resolution of 2.6 A. *J. Mol. Biol.* 134, 109–142 (1979).
ONLINE METHODS

N-terminal His6-tagged SAMHD1c2 and its mutants were expressed in Escherichia coli and purified with Ni-NTA-affinity and size-exclusion chromatography as previously described15. SAMHD1c2 and α-thio-dGTP (or the RN mutant and dGTP) were cocrySTALLized with the microbatch under-oil method. Diffraction data were collected at the Advanced Photon Source beamline 24-ID and the National Synchrotron Light Source beamline X25. The structure was solved by molecular replacement with the coordinates of SAMHD1c1 (PDB 3U1N) as a search model. Diffraction data and refinement statistics are summarized in Table 1. Inhibition of HIV-1 infection by SAMHD1 and dATP pools in U937 cells, with HIV-1 RT-based single nucleotide–incorporation assay, were quantified as described previously15,31.

Protein expression and purification. The CDNs encoding residues 1–626 and 113–626 of SAMHD1 were individually cloned into the pET28b expression vector (EMDBioscience) with a His6-tag at the N terminus. Site-directed mutants of SAMHD1 were constructed by QuikChange mutagenesis (Agilent). All proteins were expressed and purified as described previously15. In brief, SAMHD1 proteins were expressed in E. coli Rosetta 2 (DE3) cells grown in Luria–Bertani medium at 250 r.p.m., 18°C for 16 h. We prepared lysates by passing resuspended cells through a microfluidizer. Proteins were purified by Ni-NTA–affinity and size-exclusion chromatography: The proteins were stored in a buffer containing 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM DTT, 10% glycerol and 0.02% sodium azide.

Crystallization and data collection. The SAMHD1c2 mutant (H206R D207N, RN) in a solution containing 20 mM Tris-HCl, pH 7.8, 50 mM NaCl, 5 mM MgCl2, 0.02% azide and 2 mM dGTP (final concentration) and incubated at 4°C for 30 min before crystallization. Crystals were grown at 25°C with the microbatch under-oil method22 by mixture of 1 µL protein (5 mg/ml) with 1 µL crystallization buffer (100 mM SPG (Qiagen), pH 7.4, 25% PEG 1500). Crystals were improved by streak seeding. Crystals were cryoprotected by the addition of 20% (v/v) ethylene glycol. Diffraction data were collected at the National Synchrotron Light Source beamline X25 (wavelength 1.10 Å and temperature 100 K). Wild-type SAMHD1c2 was cocrySTALLized with 4 mM α-thio-dGTP (ChemCyte Incorporated) with the crystallization buffer containing 100 mM Bis-Tris, pH 6.7, and 25% (w/v) PEG 1500. Crystals were cryoprotected by 25% (v/v) glycerol. Data were collected at Advanced Photon Source beamline 24-ID-C (wavelength 0.979 Å and temperature 100 K). The data statistics are summarized in Table 1.

Structure determination and refinement. The structures were solved by molecular replacement with the previously published coordinates of SAMHD1c1 (PDB 3U1N) as a search model. Phaser23 was used for the molecular-replacement search. Four molecules were identified in an asymmetric unit of the crystal. The initial model was autobuilt by Buccaneer24 and refined by iterative rounds of TLS and noncrystallographic symmetry (NCS)-restrained refinement with Refmac5 (ref. 35) followed by manual rebuilding with Coot36. The high-resolution SAMHD1c2-RN structure was used as a reference model during the refinement of the wild-type structure. Refinement statistics are summarized in Table 1. The final models were validated by MolProbity37. The Φ-Ψ angles of 98.6% of residues of the RN structure and 98.8% of the wild-type structure lie within favored regions of Ramachandran plot, and those of all residues lie in the allowed regions.

Tetramer dissociation assays by analytical size-exclusion chromatography. SAMHD1 proteins (0.5 µM) were preincubated with dGTP (40 µM) or without dGTP, and the mixtures (50 µL) were injected to an analytical gel-filtration column (Superdex200, 10 × 300 mm) equilibrated with a buffer containing 20 mM Tris-HCl, pH 7.8, 50 mM NaCl, 10 mM dGTP, 5 mM MgCl2 and 5% glycerol, at a flow rate of 0.5 mL/min. The elution profiles (fluorescence trace of excitation at 282 nm and emission at 313 nm) were recorded. The tetramer (%) was calculated by integration of the areas containing tetramer peak and monomer/dimer peak15.

dNTP triphosphohydrolase (dNTPase) assays. Assays of SAMHD1 enzymatic activities were carried out in a reaction buffer containing 20 mM Tris-HCl, pH 7.8, 50 mM NaCl, 2 mM MgCl2, 5% glycerol, 25 µM dGTP, 1 mM dATP, 5 µM BSA and 0.1 µM recombinant SAMHD1. The amounts of products were quantified by RP-HPLC after the reaction was quenched with 50 mM of EDTA after specific time intervals, as described previously25. The average rate of dA production (nmol/(min × nmol) of SAMHD1) was calculated by linear regression analysis after plotting of the amount of dA product (on the ordinate) and the reaction time (on the abscissa).

Mammalian expression constructs and viruses. Human SAMHD1 deletion and point mutants were constructed with standard techniques and subcloned into the MSCV (puro) retrovector viral. VSV-G pseudotyped viral particles were produced from transiently transfected HEK 293T cells15. The single-cycle HIV-1 luciferase reporter construct, constructed by N. Landau58, was provided by T. Hope and D. McDonald.

SAMHD1 restriction assays. Wild-type or mutant Flag epitope–tagged SAMHD1 proteins were expressed in U937 cells (2 × 10⁶ cells) by retroviral transduction. 2 d after infection, cells were selected by supplementation of the medium with puromycin (2 µg/ml) and 1 d later were plated in 24-well plates in the presence of 100 ng/ml phorbol 12-myristate 13-acetate (PMA) for 48 h, kept for 1 d in fresh medium and then challenged with a single-cycle HIV-1 NL4-3-Luc-R-E expressing the luciferase reporter. Cell extracts were prepared from the infected cells 48 h later, and luciferase activity was quantified with the Luciferase Assay System (Promega). In dose-response experiments, cells were transduced with MSCV (puro) vectors expressing SAMHD1 at multiplicities of infection (moi) of approximately 1:1, 3:1, 10:1 and 30:1 and then selected with puromycin. The increase in the SAMHD1 levels reflects SAMHD1 expression from an increased number of integrated proviruses per cell. SAMHD1 levels in cell extracts were quantified by western blotting.

Western blotting. Cell extracts were separated by SDS-PAGE and transferred to a PVDF membrane for immunoblotting. SAMHD1 was detected with a monoclonal antibody specific for the Flag epitope tag (Sigma, F1804, 1:10,000). α-tubulin was used as a loading control was detected with an antibody purchased from Sigma-Aldrich (T5168, 1:5,000). Immune complexes were revealed with fluorescent antibodies to mouse immunoglobulin G (LI-COR, 926-32210, 1:60,000) and quantified with an Odyssey Infrared Imager (Licor). In SAMHD1 dose-response dNTPase and restriction assays, SAMHD1 fluorescent signals were normalized with those of α-tubulin as a reference. Validations of the antibodies are provided at the manufacturers’ websites.

Quantification of dATP pools in U937 cells. U937 cells were transduced with SAMHD1-expressing MSCV (puro) vectors as described above. dNTPs were extracted from PMA-differentiated U937 cells (5 × 10⁶ cells) and evaporated under vacuum at 70°C, and the lyophilized extracts were stored at ~80°C till the analysis. The dried material was resuspended in 100 µL H2O, and dATP concentration was determined with HIV-1 RT-based single nucleotide–incorporation assay, essentially as described previously31. Standard curves prepared with known amounts of dATP were used to calculate dATP amounts in test samples on the basis of the extent of primer extension, as described previously31. Original images of blots used in this study can be found in Supplementary Figure 6.

31. Diamond, T.L. et al. Macrophage tropism of HIV-1 depends on efficient cellular dNTP utilization by reverse transcriptase. J. Biol. Chem. 279, 51545–51553 (2004).
32. Chayen, N.E., Shaw Stewart, P.D., Maeder, D.L. & Blow, D.M. An automated system for micro-batch protein crystallization and screening. J. Appl. Crystallogr. 23, 297–302 (1990).
33. McCoy, A.J. et al. Phaser crystallographic software. J. Appl. Crystallogr. 40, 658–674 (2007).
34. Cowtan, K. The Buccaneer software for automated model building. 1. Tracing protein chains. Acta Crystallogr. D Biol. Crystallogr. 62, 1052–1061 (2006).
35. Vagin, A.A. et al. REFMAC5 dictionary: organization of prior chemical knowledge and guidelines for its use. Acta Crystallogr. D Biol. Crystallogr. 60, 2184–2195 (2004).
36. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132 (2004).
37. Davis, I.W. et al. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res. 35, W375–B3 (2007).
38. Connor, R.I., Chen, B.K., Choe, S. & Landau, N.R. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. Virology 206, 935–944 (1995).