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Structural basis of second-generation HIV integrase inhibitor action and viral resistance

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Despite worldwide prescription, the mechanistic basis for superiority of second-generation HIV integrase (IN) strand transfer inhibitors (INSTIs) is poorly understood. We use single-particle cryo-electron microscopy to visualize the mode of action of the advanced INSTIs dolutegravir and bictegravir at near atomic resolution. Q148H/G140S amino acid substitutions in IN that pervade clinical INSTI failure perturb optimal magnesium ion coordination in the enzyme active site. The expanded chemical scaffolds of second-generation compounds mediate interactions with the protein backbone, which are critical for antagonizing Q148H/G140S mutant virus. Our results reveal that binding to magnesium ions underpins a fundamental weakness of the INSTI pharmacophore that is exploited by the virus to engender resistance and provide a structural framework for the development of this important class of anti-HIV/AIDS therapeutics.

Despite immediate clinical impact, the first in-class INSTI raltegravir (RAL) suffered setbacks from the emergence of viral resistance (1). Although second-generation INSTIs dolutegravir (DTG) and bictegravir (BIC) display improved activity against RAL-resistant strains (2, 3), the advanced compounds are not immune to resistance (3–7). In particular, Q148H/G140S changes in HIV-1 IN are associated with complete or partial loss of efficacy across the entire drug class. The mode of INSTI binding to the IN active site was first visualized in the context of the prototype foamy virus (PFV) intasome (8). However, the limited ~15% amino acid sequence identity between PFV and HIV-1 INs greatly restricts the utility of PFV for studies of INSTI resistance and precludes its use as a template for structure-based lead optimization. Conversely, unfavorable biochemical properties of the HIV-1 intasome have impeded structural refinements to atomic resolution (9).

In order to establish a robust experimental system suitable for informing INSTI development, we evaluated IN proteins from primate lentiviruses that are highly related to circulating strains of HIV-1. The simian immunodeficiency virus from red-capped mangabeys (SIVrcm) is a direct ancestor of chimpanzee SIV (10, 11). Because the HIV-1 *pol* gene is originally derived from SIVrcm, the viruses share as much as 75% IN amino acid sequence identity (fig. S1). SIVrcm IN displayed robust strand transfer activity in vitro, which was stimulated by the lentiviral IN host factor LEDGF/p75 (12, 13). Reaction conditions were conducive for the formation of

stable nucleoprotein complexes, which were competent for strand transfer activity and sensitive to INSTI inhibition (figs. S2 and S3A). Examination of the material by negative stain electron microscopy (EM) revealed a heterogeneous population with the prominent presence of long linear polymers (hereafter referred to as stacks, Fig. 1A). Reference-free classification revealed 2D averages that were strikingly similar to those observed in maedi-visna virus (MVV) intasome preparations (Fig. 1A and fig. S4) (14). However, while the latter behaved as a near-monodispersed population with a predominance of hexadecamers (tetramer-of-tetramers) of IN, the flanking IN tetramers of SIVrcm intasomes were notably disordered, often nucleating stack formation. Although HIV-1 IN assembly was much less efficient, it yielded particles visually indistinguishable from SIVrcm intasomes (figs. S3B, S5, and S6). These observations are consistent with polydispersity previously reported in HIV-1 intasomes assembled with a hyperactive IN mutant (9). 2D class averages apparently corresponding to the dodecameric assembly from that study were readily identified in our wild type HIV-1 and SIVrcm intasome images (Fig. 1A and fig. S5).

We recorded micrograph movies of unstained SIVrcm intasome stacks in vitreous ice using a direct electron detector and refined the cryo-EM structure of an averaged intasome repeat unit. To prevent DNA binding to the target binding groove, which would occlude INSTI occupancy (15), the intasomes were prepared using A119D IN that precludes target DNA capture without affecting IN active site function (16–18).

The overall resolution of the reconstruction throughout the conserved intasome core (CIC) was 3.3 Å, while the local resolution of the active site region approached 2.8 Å (figs. S7 and S8 and table S1). In agreement with the resolution metrics, the cryo-EM density map was sufficiently detailed to build and refine an atomic model (fig. S9). The resulting model encompassed two IN tetramers with associated viral DNA ends, as well as two pairs of C- and N-terminal domains (CTDs and NTDs) donated by flanking stack units (Fig. 1, B and C). Exchange of NTDs and CTDs between neighboring intasomes forms the structural basis for stack formation (Fig. 1B and fig. S10).

Using available nucleotide sequence data (10), we engineered recombinant SIVrcm and evaluated its sensitivity to INSTIs (fig. S11A). First- (RAL) and second- (DTG, BIC) generation INSTIs inhibited HIV-1 and SIVrcm at similar 50% effective concentrations (EC_{50}) (Fig. 2A). Q148H/G140S changes in IN rendered both HIV-1 and SIVrcm >2,000-fold resistant to RAL, while EC_{50} values of the second-generation INSTIs BIC and DTG increased ~5 to 8-fold against HIV-1 and 40 to 73-fold against SIVrcm (fig. S11B). Importantly, the majority of residues that when altered confer INSTI resistance are conserved between HIV-1 and SIVrcm (fig. S1). An exception is Thr138: in HIV-1, E138T potentiates resistance of Q148H-containing viruses (19, 20). Concordantly, reverting Thr138 to Glu decreased DTG and BIC resistance of Q148H/G140S SIVrcm to the levels observed with HIV-1 Q148H/G140S. Moreover, T97A/L74M, which increase resistance of Q148H/G140S HIV-1 to second-generation INSTIs (7), exerted the same effect on SIVrcm (fig. S11B).

Encouraged by these results, we acquired cryo-EM data on SIVrcm intasomes vitrified in the presence of INSTIs and Mg^{2+} ions. DTG- and BIC-bound structures were reconstructed to resolutions of 3.0 and 2.6 Å across the CIC, with local resolutions within active site regions of 2.8 and 2.4 Å, respectively (figs. S7 and S8 and table S1). The inhibitors were defined remarkably well in density maps, allowing their refinements with bound Mg^{2+} ions and associated water molecules (figs. S12 and S13 and movie S1). The invariant IN active site carboxylates Asp64, Asp116, and Glu152 coordinate a pair of Mg^{2+} ions, which in turn interact with the metal chelating cores of the INSTIs (Fig. 2, B and C). As previously observed in PFV intasome crystals (8), the drugs displace the 3' viral DNA nucleotide, which stacks against the central body of the INSTI (fig. S14). In agreement with low-level amino acid sequence identity, there are considerable differences in the environment of the small molecules in the SIVrcm and PFV structures (fig. S15).

The map of the BIC complex revealed an interaction between the side chain amide of Gln148 and the carboxylates of metal-chelating residues Glu152 and Asp116 via a water molecule (W5, Fig. 2B). Molecular dynamics simulations

confirmed stability of this hydrogen bonding network (fig. S16A). DTG and BIC intimately contact the backbone atoms of Asn117 and Gly118 from the IN $\beta 4$ - $\alpha 2$ connector, making respectively 8 and 12 contacts with interatomic distances ≤ 5 Å. Moreover, BIC makes three contacts with interatomic distances of 3.9-4.0 Å within this region of the active site. We obtained a truncated INSTI derivative lacking the heterocycle involved in these interactions to test their importance to drug potency (analog **1**, Fig. 3A). This modification was not expected to impact the metal chelating properties of the compound or its ability to stack with DNA bases, and indeed analog **1** and DTG similarly inhibited HIV-1 infection. However, in contrast to DTG, analog **1** was ~80-fold less effective against HIV-1 Q148H/G140S (Fig. 3B). In agreement with published work (21), the amino acid substitutions increased the dissociative rate of DTG from HIV-1 intasomes, while their impact on the truncated derivative was much greater (Fig. 3A). Collectively, these data implicate contacts with the $\beta 4$ - $\alpha 2$ connector as a crucial feature of the second-generation INSTIs.

To visualize the impact of the Q148H/G140S substitutions on drug binding, we imaged mutant SIVrcm intasomes in complex with BIC to a local resolution of 2.8 Å (figs. S7, S8, and S17A and table S1). Ser140 and His148 side chains directly interact, and the latter positioned within 3.3 Å of the metal-chelating Glu152 carboxylate (Fig. 2D and fig. S17B). In the refined model, steric clashes between the side chains are avoided by a 0.5-Å shift at the His148 C α atom. Importantly, local crowding due to insertion of the mutant His148 side chain expelled water molecule W5 (fig. S16B), thus disturbing the secondary coordination shell of the Mg^{2+} ions. We also note that the amino acid changes caused a ~0.5-Å shift in the position of the bound drug; while arguably minor given the resolution of the cryo-EM map, the observed displacement agrees precisely with predictions by computational chemistry, illustrating the effect of the substitutions on drug binding. The N ϵ 2 atom of His148, which intimately contacted the carboxylate of Glu152 (3.3 Å, fig. S17), is involved in bidentate coordination with one of the Mg^{2+} atoms. Importantly, the acidity of His148 N ϵ 2 is increased due to hydrogen bonding of N δ 1 with Ser140 (Fig. 2D). The Ser140-His148-Glu152 coupling is strikingly reminiscent of the non-catalytic Ser-His-Glu triad proposed as a stability determinant in α -amylases, representing a reversal of the charge relay system in hydrolase active sites (22, 23). However, hydrogen bonding would require reorientation of IN Glu152 and His148 side chains, which would be incompatible with Mg^{2+} ion coordination and drug binding, suggesting an empirical interpretation of the INSTI resistance mechanism.

Our simulations show that analog **1** is considerably more dynamic in the active site compared to the full-sized molecule, the mobility of which is restricted through interactions

with the $\beta 4\text{-}\alpha 2$ connector (fig. S18). The additional degree of freedom is expected to allow more extensive re-orientation of the truncated inhibitor, which may permit His148 to withdraw more electron density from the Mg^{2+} -ligand cluster. Our natural bond orbital analysis illustrates the changes of atomic charge distribution within the cluster in response to polarization by protonated His148 N ϵ 2 and subtle conformational adaptations (fig. S19). It is easy to extend these observations to the other substitutions at position 148, Lys and Arg, both of which introduce electropositive functionalities to yield high-level INSTI resistance (4).

Further work will be required to unravel long-range interactions involved in boosting INSTI resistance by secondary changes such as E138T and L74M/T97A (19, 20). As a start, we analyzed respective side chains in our SIVrcm Q148H/G140S intasome structure. Thr138 is ideally positioned to hydrogen bond with N δ 1 of conserved residue His114, prompting it to donate its N ϵ 2 proton to Ser140 (Fig. 2E and fig. S20). This extended network, which may form a proton wire, is expected to reinforce Ser140 as a hydrogen bond donor for its interaction with His148 N δ 1, explaining why the E138T substitution can enhance the resistance of Q148H/G140S HIV-1 (19, 20). SIVrcm IN residues Ile74 (the position occupied by Leu or Ile in HIV-1 strains) and Thr97 are in close proximity to the side chain of conserved Phe121, which is involved in van der Waals interactions with the metal-chelating carboxylate of Asp116 (Fig. 2F and fig. S21A). Readjustment of the Phe121 side chain in response to changes in its local packing environment serves as a likely conduit to perturb the structural integrity of the metal-chelating cluster (fig. S21B).

The interactions with Mg^{2+} ions, which are nearly covalent in nature, are partly responsible for the extraordinary tight binding of INSTIs. Our results reveal that the chink in the armor of this drug class, exploited by the virus, is the extreme sensitivity of metal ions for the precise geometry and electronic properties of the ligand cluster (24, 25). Each DNA-bound IN active site within the intasome catalyzes just one strand transfer event, allowing the virus to balance INSTI resistance by detuning its active site while retaining sufficient replication capacity. However, extending the small molecules toward the IN backbone helps to stabilize optimal binding geometry and improve the resilience of the drug in the face of INSTI resistance mutations. We note that although DTG and BIC maximally extend to the $\beta 4\text{-}\alpha 2$ connector, they leave substantial free space in the IN active site, which is occupied by solute molecules in our structures (movie S1). Extension of the INSTI scaffolds to fill this space should be explored for the development of improved compounds.

REFERENCES AND NOTES

1. K. Anstett, B. Brenner, T. Mesplede, M. A. Wainberg, HIV drug resistance against strand transfer integrase inhibitors. *Retrovirology* **14**, 36 (2017). [doi:10.1186/s12977-017-0360-7](https://doi.org/10.1186/s12977-017-0360-7) Medline
2. B. A. Johns, T. Kawasuji, J. G. Weatherhead, T. Taishi, D. P. Temelkoff, H. Yoshida, T. Akiyama, Y. Taoda, H. Murai, R. Kiyama, M. Fuji, N. Tanimoto, J. Jeffrey, S. A. Foster, T. Yoshinaga, T. Seki, M. Kobayashi, A. Sato, M. N. Johnson, E. P. Garvey, T. Fujiwara, Carbamoyl pyridone HIV-1 integrase inhibitors 3. A diastereomeric approach to chiral nonracemic tricyclic ring systems and the discovery of dolutegravir (S/GSK1349572) and (S/GSK1265744). *J. Med. Chem.* **56**, 5901–5916 (2013). [doi:10.1021/jm400645w](https://doi.org/10.1021/jm400645w) Medline
3. M. Oliveira, R.-I. Ibanescu, K. Anstett, T. Mésplède, J.-P. Routy, M. A. Robbins, B. G. Brenner; Montreal Primary HIV (PHI) Cohort Study Group, Selective resistance profiles emerging in patient-derived clinical isolates with cabotegravir, bicitegravir, dolutegravir, and elvitegravir. *Retrovirology* **15**, 56 (2018). [doi:10.1186/s12977-018-0440-3](https://doi.org/10.1186/s12977-018-0440-3) Medline
4. S. J. Smith, X. Z. Zhao, T. R. Burke Jr., S. H. Hughes, Efficacies of Cabotegravir and Bicitegravir against drug-resistant HIV-1 integrase mutants. *Retrovirology* **15**, 37 (2018). [doi:10.1186/s12977-018-0420-7](https://doi.org/10.1186/s12977-018-0420-7) Medline
5. H. T. Pham, L. Labrie, I. E. A. Wijting, S. Hassounah, K. Y. Lok, I. Portna, M. E. Goring, Y. Han, C. Lungu, M. E. van der Ende, B. G. Brenner, C. A. Boucher, B. J. A. Rijnders, J. J. A. van Kampen, T. Mésplède, M. A. Wainberg, The S230R Integrase Substitution Associated With Virus Load Rebound During Dolutegravir Monotherapy Confers Low-Level Resistance to Integrase Strand-Transfer Inhibitors. *J. Infect. Dis.* **218**, 698–706 (2018). [doi:10.1093/infdis/jiy175](https://doi.org/10.1093/infdis/jiy175) Medline
6. I. E. A. Wijting, C. Lungu, B. J. A. Rijnders, M. E. van der Ende, H. T. Pham, T. Mésplède, S. D. Pas, J. J. C. Voermans, R. Schuurman, D. A. M. C. van de Vijver, P. H. M. Boers, R. A. Gruters, C. A. B. Boucher, J. J. A. van Kampen, HIV-1 Resistance Dynamics in Patients With Virologic Failure to Dolutegravir Maintenance Monotherapy. *J. Infect. Dis.* **218**, 688–697 (2018). [doi:10.1093/infdis/jiy176](https://doi.org/10.1093/infdis/jiy176) Medline
7. W. W. Zhang, P. K. Cheung, N. Oliveira, M. A. Robbins, P. R. Harrigan, A. Shahid, Accumulation of Multiple Mutations In Vivo Confers Cross-Resistance to New and Existing Integrase Inhibitors. *J. Infect. Dis.* **218**, 1773–1776 (2018). [doi:10.1093/infdis/jiy428](https://doi.org/10.1093/infdis/jiy428) Medline
8. S. Hare, S. S. Gupta, E. Valkov, A. Engelman, P. Cherepanov, Retroviral intasome assembly and inhibition of DNA strand transfer. *Nature* **464**, 232–236 (2010). [doi:10.1038/nature08784](https://doi.org/10.1038/nature08784) Medline
9. D. O. Passos, M. Li, R. Yang, S. V. Rebersburg, R. Ghirlando, Y. Jeon, N. Shkriabai, M. Kvaratskhelia, R. Craigie, D. Lyumkis, Cryo-EM structures and atomic model of the HIV-1 strand transfer complex intasome. *Science* **355**, 89–92 (2017). [doi:10.1126/science.aah5163](https://doi.org/10.1126/science.aah5163) Medline
10. S. Ahuka-Mundeke, F. Liegeois, A. Ayoub, Y. Foupouapougnini, E. Nerrienet, E. Delaporte, M. Peeters, Full-length genome sequence of a simian immunodeficiency virus (SIV) infecting a captive agile mangabey (*Cercocebus agilis*) is closely related to SIVrcm infecting wild red-capped mangabeys (*Cercocebus torquatus*) in Cameroon. *J. Gen. Virol.* **91**, 2959–2964 (2010). [doi:10.1099/vir.0.025767-0](https://doi.org/10.1099/vir.0.025767-0) Medline
11. P. M. Sharp, G. M. Shaw, B. H. Hahn, Simian immunodeficiency virus infection of chimpanzees. *J. Virol.* **79**, 3891–3902 (2005). [doi:10.1128/JVI.79.7.3891-3902.2005](https://doi.org/10.1128/JVI.79.7.3891-3902.2005) Medline
12. P. Cherepanov, LEDGF/p75 interacts with divergent lentiviral integrases and modulates their enzymatic activity in vitro. *Nucleic Acids Res.* **35**, 113–124 (2007). [doi:10.1093/nar/gkl885](https://doi.org/10.1093/nar/gkl885) Medline
13. S. Hare, M.-C. Shun, S. S. Gupta, E. Valkov, A. Engelman, P. Cherepanov, A novel co-crystal structure affords the design of gain-of-function lentiviral integrase mutants in the presence of modified PSIP1/LEDGF/p75. *PLOS Pathog.* **5**, e1000259 (2009). [doi:10.1371/journal.ppat.1000259](https://doi.org/10.1371/journal.ppat.1000259) Medline
14. A. Ballandras-Colas, D. P. Maskell, E. Serrao, J. Locke, P. Swuec, S. R. Jónsson, A. Kotecha, N. J. Cook, V. E. Pye, I. A. Taylor, V. Andrésdóttir, A. N. Engelman, A. Costa, P. Cherepanov, A supramolecular assembly mediates lentiviral DNA integration. *Science* **355**, 93–95 (2017). [doi:10.1126/science.aah7002](https://doi.org/10.1126/science.aah7002) Medline
15. A. S. Espeseth, P. Felock, A. Wolfe, M. Witmer, J. Grobler, N. Anthony, M. Egbertson, J. Y. Melamed, S. Young, T. Hamill, J. L. Cole, D. J. Hazuda, HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique

- strand transfer conformation for integrase. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 11244–11249 (2000). [doi:10.1073/pnas.200139397](https://doi.org/10.1073/pnas.200139397) [Medline](#)
16. W. M. Konsavage Jr., S. Burkholder, M. Sudol, A. L. Harper, M. Katzman, A substitution in rous sarcoma virus integrase that separates its two biologically relevant enzymatic activities. *J. Virol.* **79**, 4691–4699 (2005). [doi:10.1128/JVI.79.8.4691-4699.2005](https://doi.org/10.1128/JVI.79.8.4691-4699.2005) [Medline](#)
 17. M. G. Nowak, M. Sudol, N. E. Lee, W. M. Konsavage Jr., M. Katzman, Identifying amino acid residues that contribute to the cellular-DNA binding site on retroviral integrase. *Virology* **389**, 141–148 (2009). [doi:10.1016/j.virol.2009.04.014](https://doi.org/10.1016/j.virol.2009.04.014) [Medline](#)
 18. G. N. Maertens, S. Hare, P. Cherepanov, The mechanism of retroviral integration from X-ray structures of its key intermediates. *Nature* **468**, 326–329 (2010). [doi:10.1038/nature09517](https://doi.org/10.1038/nature09517) [Medline](#)
 19. R. W. Shafer, Rationale and uses of a public HIV drug-resistance database. *J. Infect. Dis.* **194** (Suppl. 1), S51–S58 (2006). [doi:10.1086/505356](https://doi.org/10.1086/505356) [Medline](#)
 20. J. M. George, S. S. Kuriakose, N. Dee, P. Stoll, T. Lalani, R. Dewar, M. A. Khan, M. T. Rehman, Z. Grossman, F. Maldarelli, A. K. Pau, Rapid Development of High-Level Resistance to Dolutegravir With Emergence of T97A Mutation in 2 Treatment-Experienced Individuals With Baseline Partial Sensitivity to Dolutegravir. *Open Forum Infect. Dis.* **5**, ofy221 (2018). [doi:10.1093/ofid/ofy221](https://doi.org/10.1093/ofid/ofy221) [Medline](#)
 21. K. E. Hightower, R. Wang, F. Deanda, B. A. Johns, K. Weaver, Y. Shen, G. H. Tomberlin, H. L. Carter 3rd, T. Broderick, S. Sigethy, T. Seki, M. Kobayashi, M. R. Underwood, Dolutegravir (S/GSK1349572) exhibits significantly slower dissociation than raltegravir and elvitegravir from wild-type and integrase inhibitor-resistant HIV-1 integrase-DNA complexes. *Antimicrob. Agents Chemother.* **55**, 4552–4559 (2011). [doi:10.1128/AAC.00157-11](https://doi.org/10.1128/AAC.00157-11) [Medline](#)
 22. D. Blow, More of the catalytic triad. *Nature* **343**, 694–695 (1990). [doi:10.1038/343694a0](https://doi.org/10.1038/343694a0) [Medline](#)
 23. J. C. Marx, J. Poncin, J. P. Simorre, P. W. Ramteke, G. Feller, The noncatalytic triad of α -amylases: A novel structural motif involved in conformational stability. *Proteins* **70**, 320–328 (2008). [doi:10.1002/prot.21594](https://doi.org/10.1002/prot.21594) [Medline](#)
 24. M. E. Maguire, J. A. Cowan, Magnesium chemistry and biochemistry. *Biomaterials* **15**, 203–210 (2002). [doi:10.1023/A:1016058229972](https://doi.org/10.1023/A:1016058229972) [Medline](#)
 25. M. M. Harding, Geometry of metal-ligand interactions in proteins. *Acta Crystallogr. D* **57**, 401–411 (2001). [doi:10.1107/S0907444900019168](https://doi.org/10.1107/S0907444900019168) [Medline](#)
 26. F. Turlure, G. Maertens, S. Rahman, P. Cherepanov, A. Engelman, A tripartite DNA-binding element, comprised of the nuclear localization signal and two AT-hook motifs, mediates the association of LEDGF/p75 with chromatin in vivo. *Nucleic Acids Res.* **34**, 1653–1665 (2006). [doi:10.1093/nar/gkl052](https://doi.org/10.1093/nar/gkl052) [Medline](#)
 27. S. Q. Zheng, E. Palovcak, J. P. Armache, K. A. Verba, Y. Cheng, D. A. Agard, MotionCor2: Anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat. Methods* **14**, 331–332 (2017). [doi:10.1038/nmeth.4193](https://doi.org/10.1038/nmeth.4193) [Medline](#)
 28. K. Zhang, Gctf: Real-time CTF determination and correction. *J. Struct. Biol.* **193**, 1–12 (2016). [doi:10.1016/j.jsb.2015.11.003](https://doi.org/10.1016/j.jsb.2015.11.003) [Medline](#)
 29. G. Tang, L. Peng, P. R. Baldwin, D. S. Mann, W. Jiang, I. Rees, S. J. Ludtke, EMAN2: An extensible image processing suite for electron microscopy. *J. Struct. Biol.* **157**, 38–46 (2007). [doi:10.1016/j.jsb.2006.05.009](https://doi.org/10.1016/j.jsb.2006.05.009) [Medline](#)
 30. D. Kimanius, B. O. Forsberg, S. H. Scheres, E. Lindahl, Accelerated cryo-EM structure determination with parallelisation using GPUs in RELION-2. *eLife* **5**, e18722 (2016). [doi:10.7554/eLife.18722](https://doi.org/10.7554/eLife.18722) [Medline](#)
 31. A. Punjani, J. L. Rubinstein, D. J. Fleet, M. A. Brubaker, cryoSPARC: Algorithms for rapid unsupervised cryo-EM structure determination. *Nat. Methods* **14**, 290–296 (2017). [doi:10.1038/nmeth.4169](https://doi.org/10.1038/nmeth.4169) [Medline](#)
 32. S. L. Ilca, A. Kotecha, X. Sun, M. M. Poranen, D. I. Stuart, J. T. Huiskonen, Localized reconstruction of subunits from electron cryomicroscopy images of macromolecular complexes. *Nat. Commun.* **6**, 8843 (2015). [doi:10.1038/ncomms9843](https://doi.org/10.1038/ncomms9843) [Medline](#)
 33. J. B. Heymann, Guidelines for using Bsoft for high resolution reconstruction and validation of biomolecular structures from electron micrographs. *Protein Sci.* **27**, 159–171 (2018). [doi:10.1002/pro.3293](https://doi.org/10.1002/pro.3293) [Medline](#)
 34. P. B. Rosenthal, R. Henderson, Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J. Mol. Biol.* **333**, 721–745 (2003). [doi:10.1016/j.jmb.2003.07.013](https://doi.org/10.1016/j.jmb.2003.07.013) [Medline](#)
 35. S. H. Scheres, S. Chen, Prevention of overfitting in cryo-EM structure determination. *Nat. Methods* **9**, 853–854 (2012). [doi:10.1038/nmeth.2115](https://doi.org/10.1038/nmeth.2115) [Medline](#)
 36. Y. Z. Tan, P. R. Baldwin, J. H. Davis, J. R. Williamson, C. S. Potter, B. Carragher, D. Lyumkis, Addressing preferred specimen orientation in single-particle cryo-EM through tilting. *Nat. Methods* **14**, 793–796 (2017). [doi:10.1038/nmeth.4347](https://doi.org/10.1038/nmeth.4347) [Medline](#)
 37. J. C. Chen, J. Krucinski, L. J. Miercke, J. S. Finer-Moore, A. H. Tang, A. D. Leavitt, R. M. Stroud, Crystal structure of the HIV-1 integrase catalytic core and C-terminal domains: A model for viral DNA binding. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 8233–8238 (2000). [doi:10.1073/pnas.150220297](https://doi.org/10.1073/pnas.150220297) [Medline](#)
 38. J. Y. Wang, H. Ling, W. Yang, R. Craigie, Structure of a two-domain fragment of HIV-1 integrase: Implications for domain organization in the intact protein. *EMBO J.* **20**, 7333–7343 (2001). [doi:10.1093/emboj/20.24.7333](https://doi.org/10.1093/emboj/20.24.7333) [Medline](#)
 39. P. Cherepanov, A. L. Ambrosio, S. Rahman, T. Ellenberger, A. Engelman, Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 17308–17313 (2005). [doi:10.1073/pnas.0506924102](https://doi.org/10.1073/pnas.0506924102) [Medline](#)
 40. P. Emsley, K. Cowtan, Coot: Model-building tools for molecular graphics. *Acta Crystallogr. D* **60**, 2126–2132 (2004). [doi:10.1107/S0907444904019158](https://doi.org/10.1107/S0907444904019158) [Medline](#)
 41. P. V. Afonine, B. K. Poon, R. J. Read, O. V. Sobolev, T. C. Terwilliger, A. Urzhumtsev, P. D. Adams, Real-space refinement in PHENIX for cryo-EM and crystallography. *Acta Crystallogr. D* **74**, 531–544 (2018). [doi:10.1107/S2059798318006551](https://doi.org/10.1107/S2059798318006551) [Medline](#)
 42. C. J. Williams, J. J. Headd, N. W. Moriarty, M. G. Prisant, L. L. Videau, L. N. Deis, V. Verma, D. A. Keedy, B. J. Hintze, V. B. Chen, S. Jain, S. M. Lewis, W. B. Arendall 3rd, J. Snoeyink, P. D. Adams, S. C. Lovell, J. S. Richardson, D. C. Richardson, MolProbity: More and better reference data for improved all-atom structure validation. *Protein Sci.* **27**, 293–315 (2018). [doi:10.1002/pro.3330](https://doi.org/10.1002/pro.3330) [Medline](#)
 43. B. A. Barad, N. Echols, R. Y. Wang, Y. Cheng, F. DiMaio, P. D. Adams, J. S. Fraser, EMRinger: Side chain-directed model and map validation for 3D cryo-electron microscopy. *Nat. Methods* **12**, 943–946 (2015). [doi:10.1038/nmeth.3541](https://doi.org/10.1038/nmeth.3541) [Medline](#)
 44. Y. Koh, K. A. Matreyek, A. Engelman, Differential sensitivities of retroviruses to integrase strand transfer inhibitors. *J. Virol.* **85**, 3677–3682 (2011). [doi:10.1128/JVI.02541-10](https://doi.org/10.1128/JVI.02541-10) [Medline](#)
 45. M. C. Shun, J. E. Daigle, N. Vandegraaff, A. Engelman, Wild-type levels of human immunodeficiency virus type 1 infectivity in the absence of cellular emerin protein. *J. Virol.* **81**, 166–172 (2007). [doi:10.1128/JVI.01953-06](https://doi.org/10.1128/JVI.01953-06) [Medline](#)
 46. W. Li, M. H. Lee, L. Henderson, R. Tyagi, M. Bachani, J. Steiner, E. Campanac, D. A. Hoffman, G. von Geldern, K. Johnson, D. Maric, H. D. Morris, M. Lentz, K. Pak, A. Mammen, L. Ostrow, J. Rothstein, A. Nath, Human endogenous retrovirus-K contributes to motor neuron disease. *Sci. Transl. Med.* **7**, 307ra153 (2015). [doi:10.1126/scitranslmed.aac8201](https://doi.org/10.1126/scitranslmed.aac8201) [Medline](#)
 47. D. R. Langley, H. K. Samanta, Z. Lin, M. A. Walker, M. R. Krystal, I. B. Dicker, The terminal (catalytic) adenosine of the HIV LTR controls the kinetics of binding and dissociation of HIV integrase strand transfer inhibitors. *Biochemistry* **47**, 13481–13488 (2008). [doi:10.1021/bi801372d](https://doi.org/10.1021/bi801372d) [Medline](#)
 48. J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, Development and testing of a general amber force field. *J. Comput. Chem.* **25**, 1157–1174 (2004). [doi:10.1002/jcc.20035](https://doi.org/10.1002/jcc.20035) [Medline](#)
 49. C. M. Breneman, K. B. Wiberg, Determining atom-centered monopoles from molecular electrostatic potentials. The need for high sampling density in formamide conformational analysis. *J. Comput. Chem.* **11**, 361–373 (1990). [doi:10.1002/jcc.540110311](https://doi.org/10.1002/jcc.540110311) [Medline](#)
 50. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda et al., Gaussian 09, Revision E.01.
 51. J. D. Chai, M. Head-Gordon, Long-range corrected hybrid density functionals with damped atom-atom dispersion corrections. *Phys. Chem. Chem. Phys.* **10**, 6615–6620 (2008). [doi:10.1039/b810189b](https://doi.org/10.1039/b810189b) [Medline](#)
 52. F. Weigend, R. Ahlrichs, Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. *Phys. Chem. Chem. Phys.* **7**, 3297–3305 (2005). [doi:10.1039/b508541a](https://doi.org/10.1039/b508541a) [Medline](#)

53. J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kalé, K. Schulten, Scalable molecular dynamics with NAMD. *J. Comput. Chem.* **26**, 1781–1802 (2005). [doi:10.1002/jcc.20289](https://doi.org/10.1002/jcc.20289) [Medline](#)
54. R. B. Best, X. Zhu, J. Shim, P. E. Lopes, J. Mittal, M. Feig, A. D. Mackerell Jr., Optimization of the additive CHARMM all-atom protein force field targeting improved sampling of the backbone ϕ , ψ and side-chain $\chi(1)$ and $\chi(2)$ dihedral angles. *J. Chem. Theory Comput.* **8**, 3257–3273 (2012). [doi:10.1021/ct300400x](https://doi.org/10.1021/ct300400x) [Medline](#)
55. A. D. Becke, Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **98**, 5648–5652 (1993). [doi:10.1063/1.464913](https://doi.org/10.1063/1.464913)
56. R. Ditchfield, W. J. Hehre, J. A. Pople, P. J. A., Self-Consistent Molecular-Orbital Methods. IX. An Extended Gaussian-Type Basis for Molecular-Orbital Studies of Organic Molecules. *J. Chem. Phys.* **54**, 724–728 (1971). [doi:10.1063/1.1674902](https://doi.org/10.1063/1.1674902)
57. W. J. Hehre, R. Ditchfield, J. A. Pople, Self-Consistent Molecular Orbital Methods. XII. Further Extensions of Gaussian-Type Basis Sets for Use in Molecular Orbital Studies of Organic Molecules. *J. Chem. Phys.* **56**, 2257–2261 (1972). [doi:10.1063/1.1677527](https://doi.org/10.1063/1.1677527)
58. M. M. Francl, W. J. Pietro, W. J. Hehre, J. S. Binkley, M. S. Gordon, D. J. DeFrees, J. A. Pople, Self-consistent molecular orbital methods. XXIII. A polarization-type basis set for second-row elements. *J. Chem. Phys.* **77**, 3654–3665 (1982). [doi:10.1063/1.444267](https://doi.org/10.1063/1.444267)
59. B. R. Brooks, C. L. Brooks 3rd, A. D. Mackerell Jr., L. Nilsson, R. J. Petrella, B. Roux, Y. Won, G. Archontis, C. Bartels, S. Boresch, A. Cafilisch, L. Caves, Q. Cui, A. R. Dinner, M. Feig, S. Fischer, J. Gao, M. Hodoscek, W. Im, K. Kucera, T. Lazaridis, J. Ma, V. Ovchinnikov, E. Paci, R. W. Pastor, C. B. Post, J. Z. Pu, M. Schaefer, B. Tidor, R. M. Venable, H. L. Woodcock, X. Wu, W. Yang, D. M. York, M. Karplus, CHARMM: The biomolecular simulation program. *J. Comput. Chem.* **30**, 1545–1614 (2009). [doi:10.1002/jcc.21287](https://doi.org/10.1002/jcc.21287) [Medline](#)
60. Y. Shao, Z. Gan, E. Epifanovsky, A. T. B. Gilbert, M. Wormit, J. Kussmann, A. W. Lange, A. Behn, J. Deng, X. Feng, D. Ghosh, M. Goldey, P. R. Horn, L. D. Jacobson, I. Kaliman, R. Z. Khaliullin, T. Kus, A. Landau, J. Liu, E. I. Proynov, Y. M. Rhee, R. M. Richard, M. A. Rohrdanz, R. P. Steele, E. J. Sundstrom, H. L. Woodcock III, P. M. Zimmerman, D. Zuev, B. Albrecht, E. Alguire, B. Austin, G. J. O. Beran, Y. A. Bernard, E. Berquist, K. Brandhorst, K. B. Bravaya, S. T. Brown, D. Casanova, C.-M. Chang, Y. Chen, S. H. Chien, K. D. Closser, D. L. Crittenden, M. Diedenhofen, R. A. DiStasio Jr., H. Do, A. D. Dutoi, R. G. Edgar, S. Fatehi, L. Fusti-Molnar, A. Ghysels, A. Golubeva-Zadorozhnaya, J. Gomes, M. W. D. Hanson-Heine, P. H. P. Harbach, A. W. Hauser, E. G. Hohenstein, Z. C. Holden, T.-C. Jagau, H. Ji, B. Kaduk, K. Khistyayev, J. Kim, J. Kim, R. A. King, P. Klunzinger, D. Kosenkov, T. Kowalczyk, C. M. Krauter, K. U. Lao, A. D. Laurent, K. V. Lawler, S. V. Levchenko, C. Y. Lin, F. Liu, E. Livshits, R. C. Lochan, A. Luenser, P. Manohar, S. F. Manzer, S.-P. Mao, N. Mardirossian, A. V. Marenich, S. A. Maurer, N. J. Mayhall, E. Neuscamman, C. M. Oana, R. Olivares-Amaya, D. P. O'Neill, J. A. Parkhill, T. M. Perrine, R. Peverati, A. Prociuk, D. R. Rehn, E. Rosta, N. J. Russ, S. M. Sharada, S. Sharma, D. W. Small, A. Sodt, T. Stein, D. Stück, Y.-C. Su, A. J. W. Thom, T. Tsuchimochi, V. Vanovschi, L. Vogt, O. Vydrov, T. Wang, M. A. Watson, J. Wenzel, A. White, C. F. Williams, J. Yang, S. Yeganeh, S. R. Yost, Z.-Q. You, I. Y. Zhang, X. Zhang, Y. Zhao, B. R. Brooks, G. K. L. Chan, D. M. Chipman, C. J. Cramer, W. A. Goddard III, M. S. Gordon, W. J. Hehre, A. Klamt, H. F. Schaefer III, M. W. Schmidt, C. D. Sherrill, D. G. Truhlar, A. Warshel, X. Xu, A. Aspuru-Guzik, R. Baer, A. T. Bell, N. A. Besley, J.-D. Chai, A. Dreuw, B. D. Dunietz, T. R. Furlani, S. R. Gwaltney, C.-P. Hsu, Y. Jung, J. Kong, D. S. Lambrecht, W. Liang, C. Ochsenfeld, V. A. Rassolov, L. V. Slipchenko, J. E. Subotnik, T. Van Voorhis, J. M. Herbert, A. I. Krylov, P. M. W. Gill, M. Head-Gordon, Advances in molecular quantum chemistry contained in the Q-Chem 4 program package. *Mol. Phys.* **113**, 184–215 (2015). [doi:10.1080/00268976.2014.952696](https://doi.org/10.1080/00268976.2014.952696)
61. C. M. Smith, G. G. Hall, The approximation of electron densities. *Theor. Chim. Acta* **69**, 63–69 (1986). [doi:10.1007/BF00526293](https://doi.org/10.1007/BF00526293)
62. A. E. Reed, R. B. Weinstock, F. Weinhold, Natural population analysis. *J. Chem. Phys.* **83**, 735–746 (1985). [doi:10.1063/1.449486](https://doi.org/10.1063/1.449486)
63. A. E. Reed, L. A. Curtiss, F. Weinhold, Intermolecular interactions from a natural bond orbital, donor-acceptor viewpoint. *Chem. Rev.* **88**, 899–926 (1988). [doi:10.1021/cr00088a005](https://doi.org/10.1021/cr00088a005)
64. X. Robert, P. Gouet, Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.* **42**, W320–W324 (2014). [doi:10.1093/nar/gku316](https://doi.org/10.1093/nar/gku316) [Medline](#)

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S27

Table S1

References (26–64)

Movie S1

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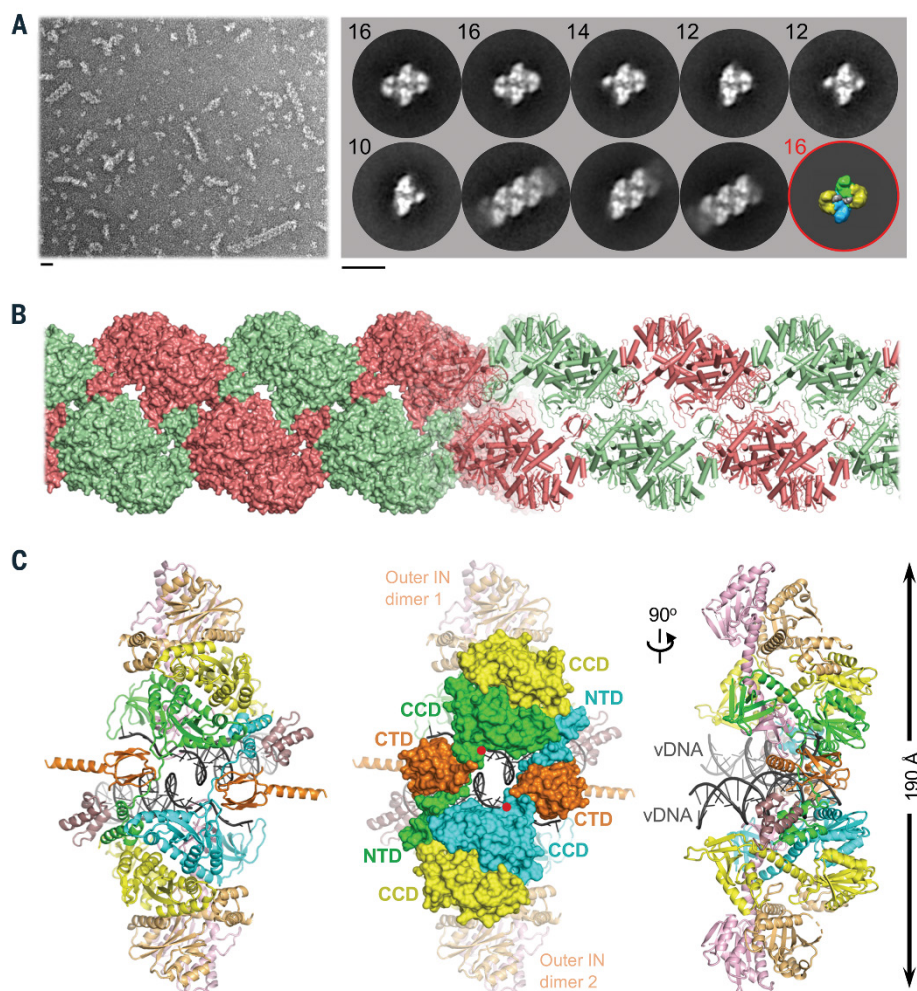


Fig. 1. Reconstruction of the SIVrcm intasome core. (A) Raw image (left) and 2D class averages (right) of negatively stained SIVrcm intasome particles; apparent numbers of IN subunits are indicated for non-stacked classes. Particle distributions are given in fig. S4. The envelope of the hexadecameric maedi-visna virus intasome (red circle; central and flanking IN tetramers in blue/green and yellow, respectively) is shown for comparison; scale bars are 0.2 nm. (B) Atomistic reconstruction of the SIVrcm intasome stack shown as space fill (left) and cartoons (right); separate repeat units are shown in alternating red and green colors. (C) Detailed view of a single intasomal repeat representing a pair of viral DNA ends (vDNA, gray cartoons) synapsed between a pair of IN tetramers (composed of yellow, orange, pink, and either green or cyan IN protomers; the active sites of the green and cyan molecules (red dots) catalyze DNA recombination). The repeat unit is completed by pairs of C-terminal (orange) and N-terminal (dark magenta) domains donated by IN chains belonging to neighboring repeats. These CTDs are critical to form the conserved intasome core (CIC), which is shown in space fill mode in the middle panel. CCD, catalytic core domain.

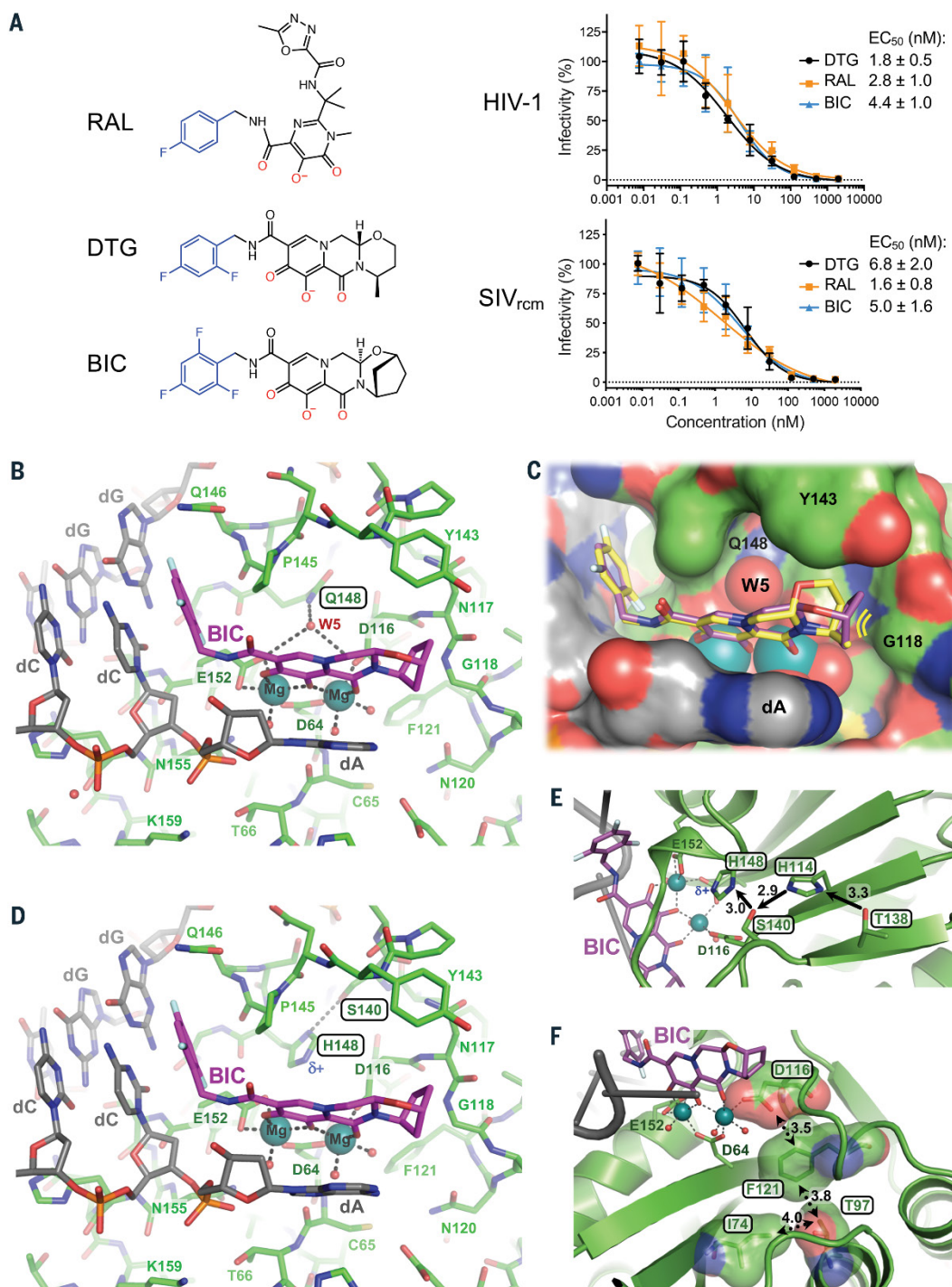


Fig. 2. Binding modes of second generation INSTIs in the IN active site. (A) Chemical structures of select first- (RAL) and second-generation (DTG, BIC) INSTIs (left; halo-benzyl groups in blue and metal-chelating oxygen atoms in red) and viral sensitivities (right). Results are averages and standard deviations of minimally $n = 2$ experiments, with each experiment conducted in triplicate; EC₅₀ values are noted. (B) Active site of the SIV_{rcm} intasome in complex with BIC; protein, DNA, and drug are shown as sticks. Blue spheres are Mg²⁺ ions, water molecules are shown as small red spheres. (C) Superposition of BIC (magenta) and DTG (yellow) bound structures with protein and DNA shown in space-fill mode. Yellow lines accentuate proximity to IN β_4 - α_2 connector. (D) Q148H/G140S active site bound to BIC. δ^+ indicates increased electropositivity of the His148 N ϵ_2 proton. (E) The extended hydrogen bond network that couples Thr138 to His148 in the Q148H/G140S SIV_{rcm} intasome. Black arrows indicate hydrogen bond donation; the corresponding interatomic distances are given in Ångstroms. Cryo-EM map of the same region is shown in fig. S20. (F) Long-range interactions of Ile74 and Thr97 with the metal chelating cluster via Phe121. Key amino acid residues are shown as sticks and semi-transparent van der Waals surfaces. Contacts between side chain atoms are indicated by double-headed dotted arrows with distances given in Ångstroms. Cryo-EM map showing definition of the side chain rotamers and the effects of I74M/T97A substitutions on the Phe121 side chain are shown in fig. S21.

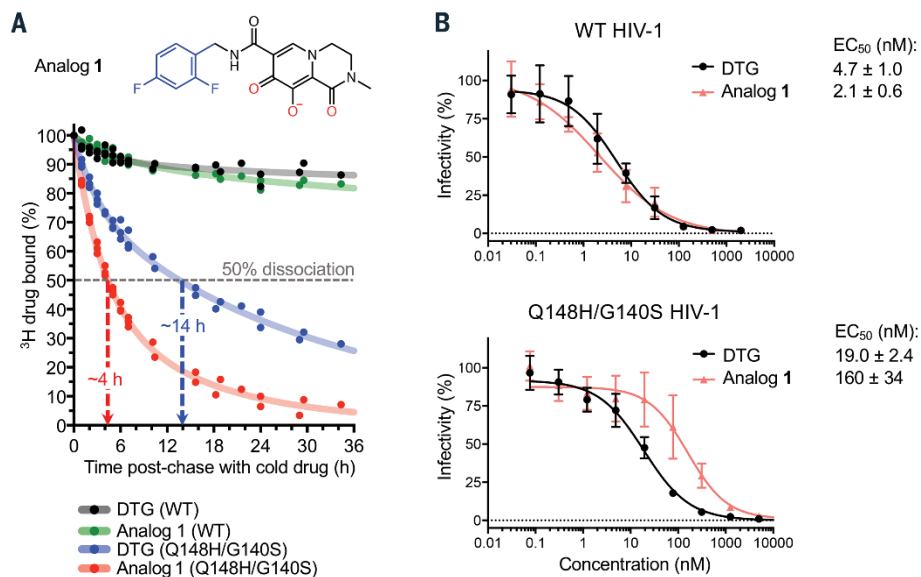


Fig. 3. Effects of Q148H/G140S substitutions on DTG and analog 1 activities. (A) Structure of analog 1 (top; colors as in Fig. 2A) and a time course of ³H-DTG and analog 1 dissociation from wild type and Q148H/G140S HIV-1 intasomes (bottom). Results from three independent experiments are plotted; each data point is an average of two measurements done in parallel; trendlines are for illustration purpose. Apparent INSTI dissociative half-times from the mutant intasome are indicated. (B) Activities of DTG and analog 1 against wild type (top) and Q148H/G140S (bottom) HIV-1. Results are averages and standard deviations of two independent experiments, with each experiment conducted in triplicate.

Structural basis of second-generation HIV integrase inhibitor action and viral resistance

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