High-resolution crystal structure of LINE-1 Reverse Transcriptase and Its Implications for Rational Inhibitor Design

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Background

The LINE-1 (L1) retrotransposon has written almost half of the human genome through a “copy-and-paste” mechanism catalyzed by its multifunctional enzyme ORF2p, which contains a reverse transcriptase (RT) domain at its core. LINE-1 RT has emerged as a potential therapeutic target implicated in of cancer, autoimmunity, and aging. However, a lack of structural and mechanistic knowledge hampers efforts to rationally exploit it. Here, we report the first high-resolution crystal structure of the human LINE-1 RT bound to a template-primer duplex and incoming deoxynucleotide triphosphate (dNTP) and characterize its inhibition profile by existing RT inhibitors.

LINE-1 Reverse Transcriptase (RT) structure and its relationship to other RT domains

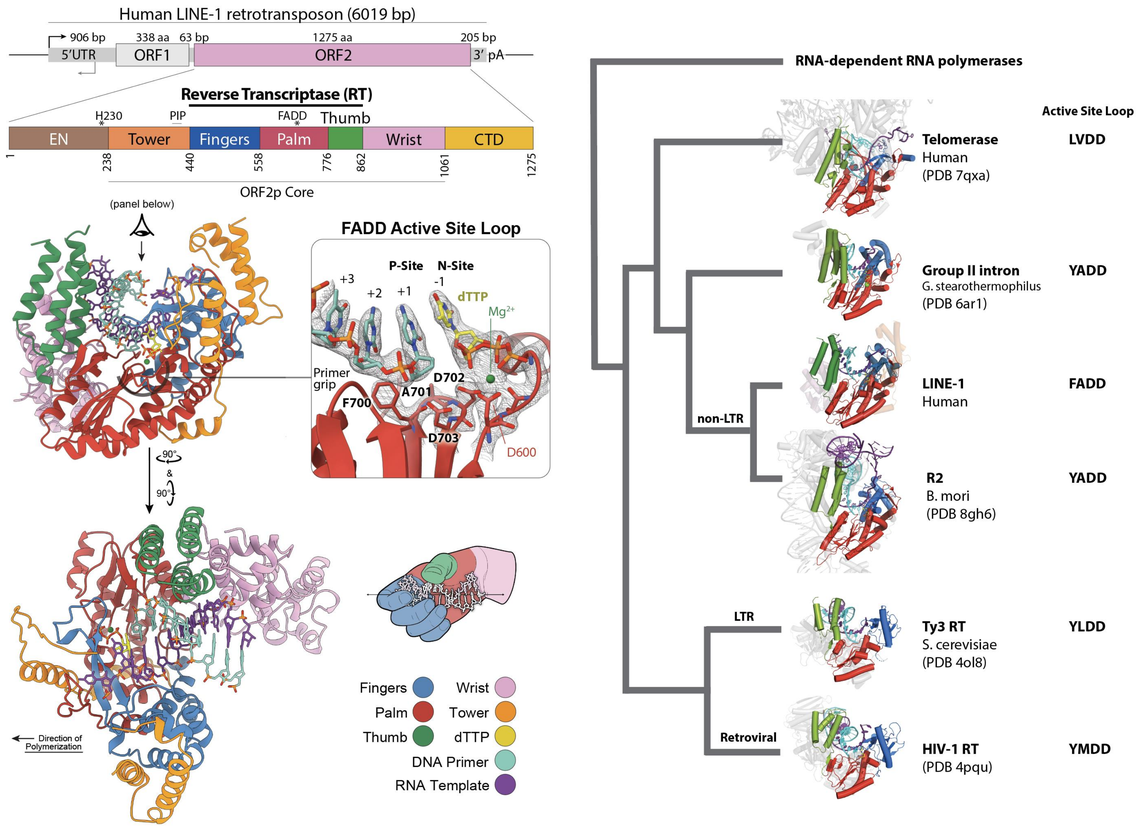


Figure 1. (Left) Structure of human LINE-1 ORF2p core containing the Reverse Transcriptase domain. (Right) Comparison of evolutionarily related RT domains of known structure (tree adapted from Xiong & Eickbush, 1990).

LINE-1 RT is not inhibited by HIV-1 Non-Nucleoside RT inhibitors (NNRTi)

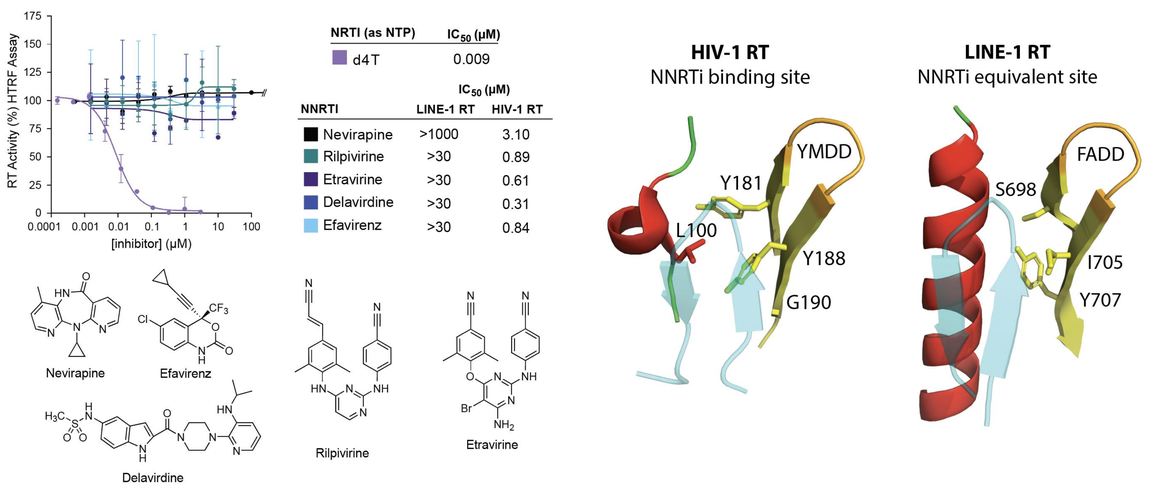
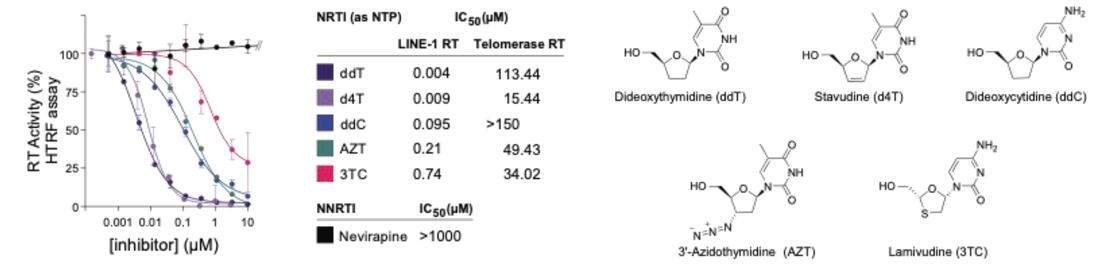
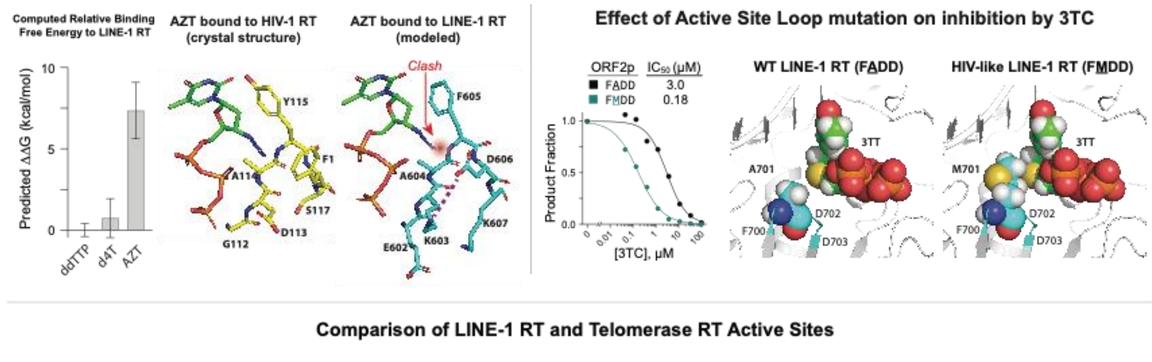


Figure 2. (Left) Inhibition of LINE-1 RT by HIV-1 NNRTis. (Right) Comparison of HIV-1 NNRTI binding site and the equivalent region in LINE-1 RT. The HIV-1 NNRTi site is not conserved in LINE-1 RT.

LINE-1 RT inhibition by Nucleoside RT inhibitors (NRTi) differs from HIV-1 RT and Telomerase





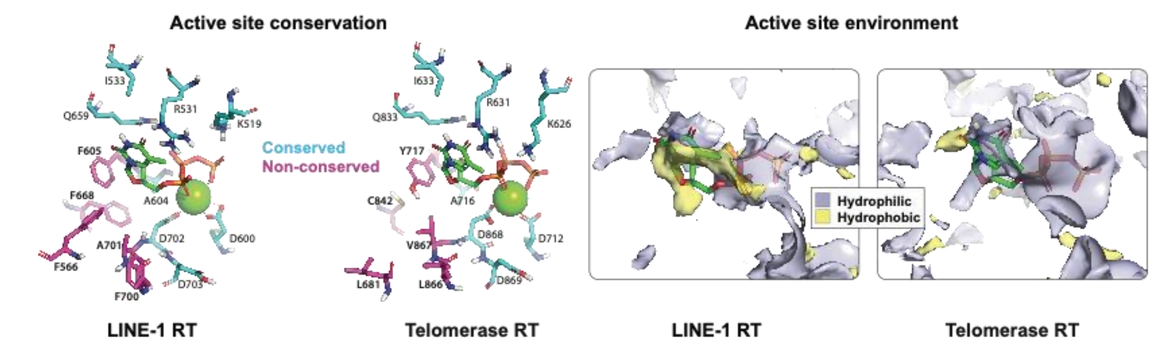


Figure 3. (**Top**) inhibition of LINE-1 RT by HIV-1 NRTis. (Middle Left) Relative Free Energy calculations and modeling explain the observed low inhibition by AZT. (Middle Right) Effect of A701M mutation on inhibition by 3TC and modeling of T-analog of 3TC. (Bottom) Comparison of LINE-1 RT and Telomerase RT active sites explains marked difference in inhibition by HIV-1 NRTis.

Results

- The LINE-1 RT crystal structure confirms the closer evolutionary relationship of non-LTR retroelement RTs with bacterial RTs and reveals marked differences with retroviral and LTR retroelement RTs.
- Characterization of LINE-1 RT inhibition by nucleoside and non-nucleoside HIV-1 RT inhibitors reveals a unique inhibition profile for LINE-1 RT, showing lower inhibition by some potent HIV-1 inhibitors such as AZT and 3TC, and no inhibition by non-nucleoside RT inhibitors.
- Computational modeling reveals that the crystal structure can provide qualitative and quantitative explanations for the differences in inhibition between LINE-1 RT and HIV-1 RT, as well as the global differences in inhibition between LINE-1 RT and Telomerase RT.

Conclusion

The determination of the first high resolution crystal structure of LINE-1 Reverse Transcriptase and testing of HIV-1 inhibitors against it reveals that LINE-1 RT has structural properties that translate into a unique inhibition profile that differs from HIV-1 RT. Development of LINE-1 RT specific inhibitors will require a dedicated effort that is enabled by the newly determined crystal structure.