# Elucidating the molecular interactions of the *L*-nucleotide analogs with the complex of HIV-1 reverse transcriptase and DNA and the mechanism of drug resistance conferred by the M184V mutation

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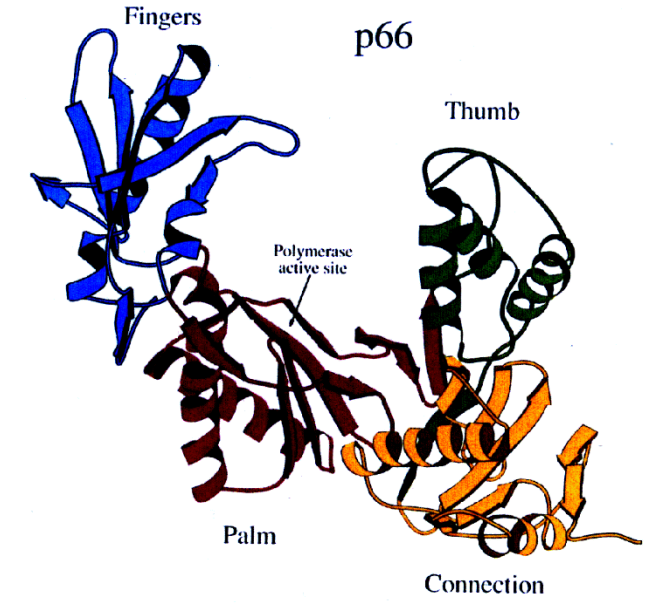
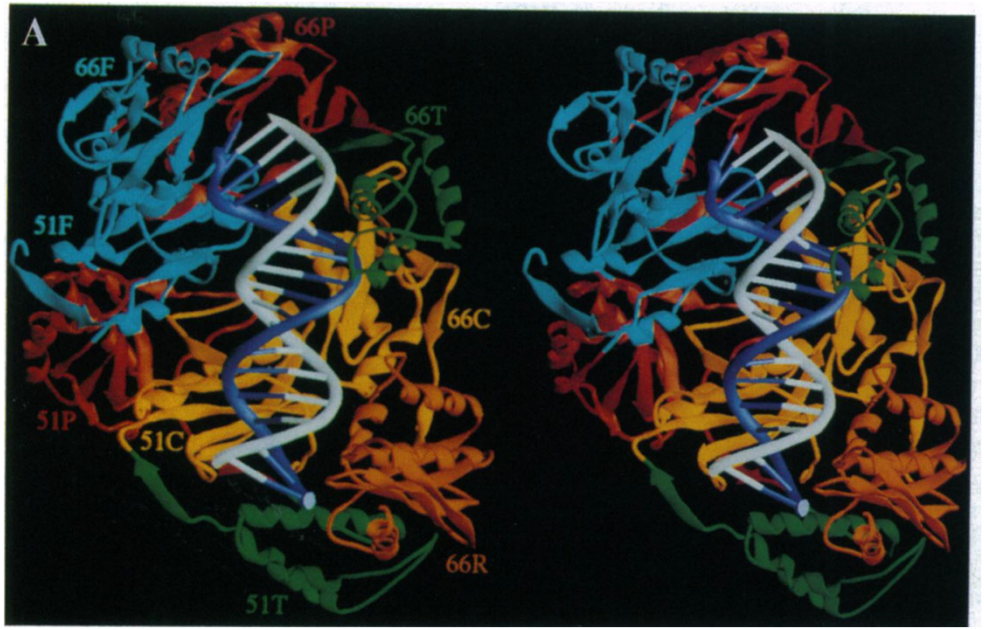
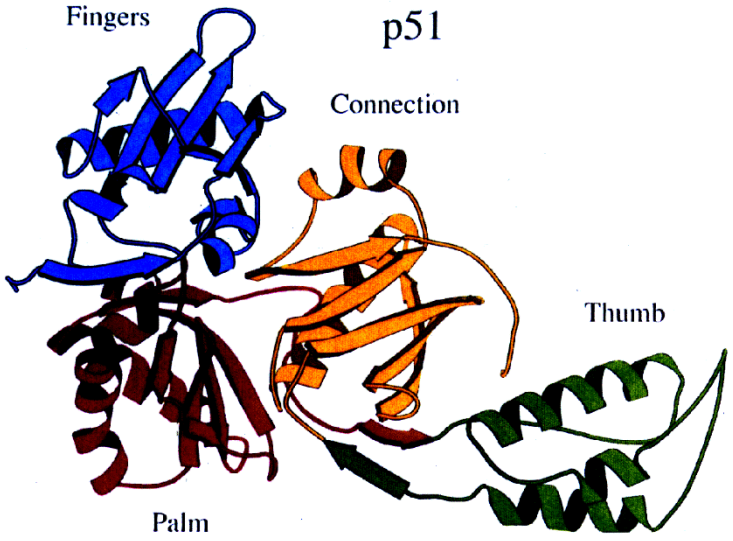
# Crystal structures of p66 and p51 subunits in HIV-1 RT

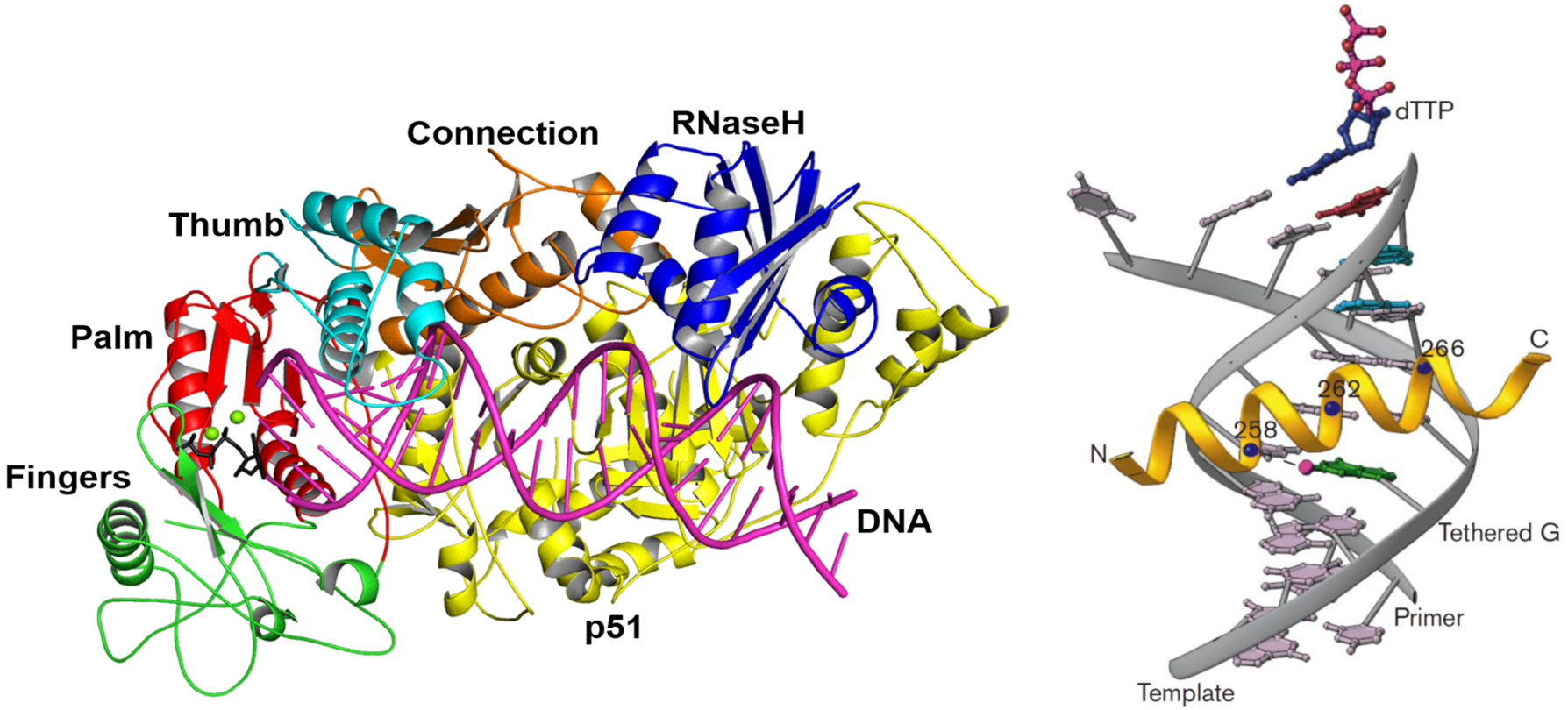


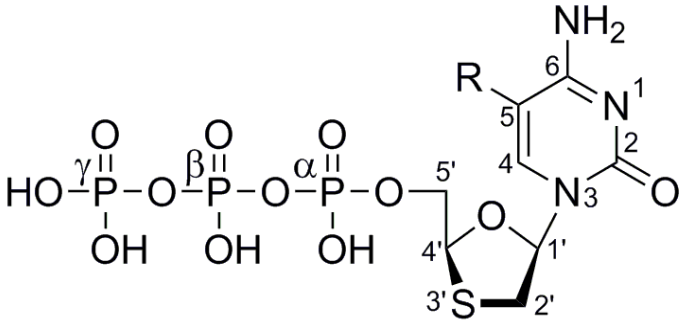

Jacobo-Monlina *et al.*, 1994, *Proc. Natl. Acad. Sci. USA*, 90, 6320-6324

# Ternary crystal structure of HIV-1 RT-dideoxyDNA•dTTP

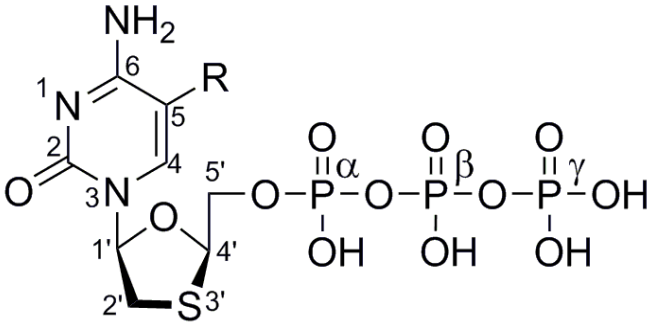



Huang, H., Chopra, R., Verdine, G.L. & Harrison, S.C. *Science* **282**, 1669-75 (1998).

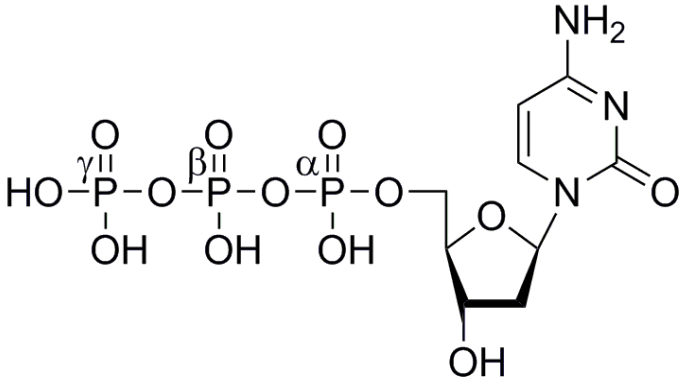
mirror



R = H, (+)-3TC-TP
R = F, (+)-FTC-TP



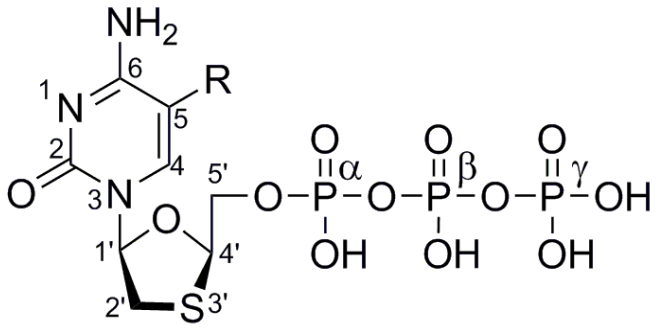
R = H, (-)-3TC-TP
R = F, (-)FTC-TP



dCTP

(-)3TC: Lamivudine

(-)FTC: Emtricitabine



R = H, (-)-3TC-TP
R = F, (-)-FTC-TP

- Structural basis for the binding and incorporation of *L*-nucleotide analogs by HIV-1 RT

- Mechanistic basis for >500 fold clinical resistance to (-)FTC and (-)3TC via M184V in HIV-1 RT

## DNA: 18/26-mer

5′-GTCCCTGTTCGGGCGCCC-3′

3′-TGTCAGGGACAAGCCCGCGGGGAGTA-5′

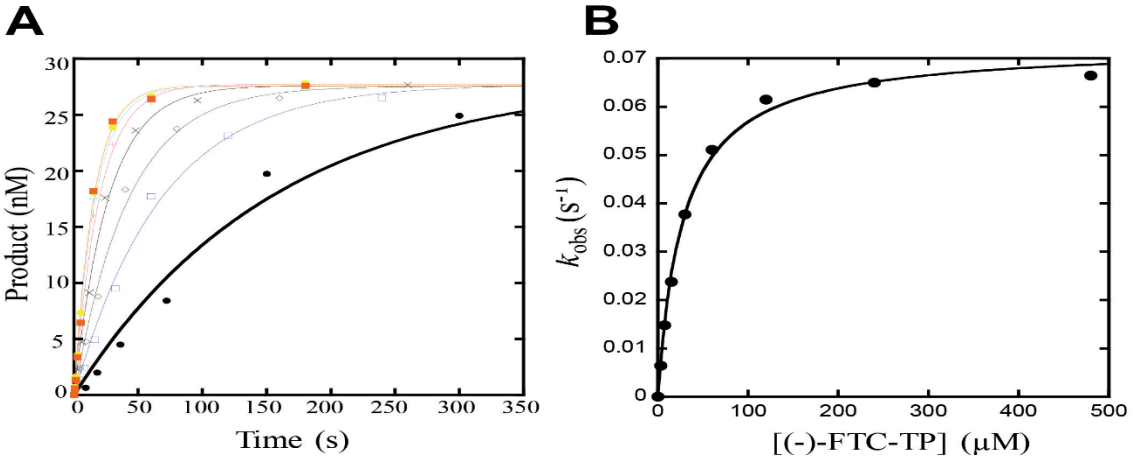



**Table 1 Pre-steady-state kinetic parameters for incorporation of dCTP, FTC-TP, and 3TC-TP by WT or M184V HIV-1 RT at 37 °C.**

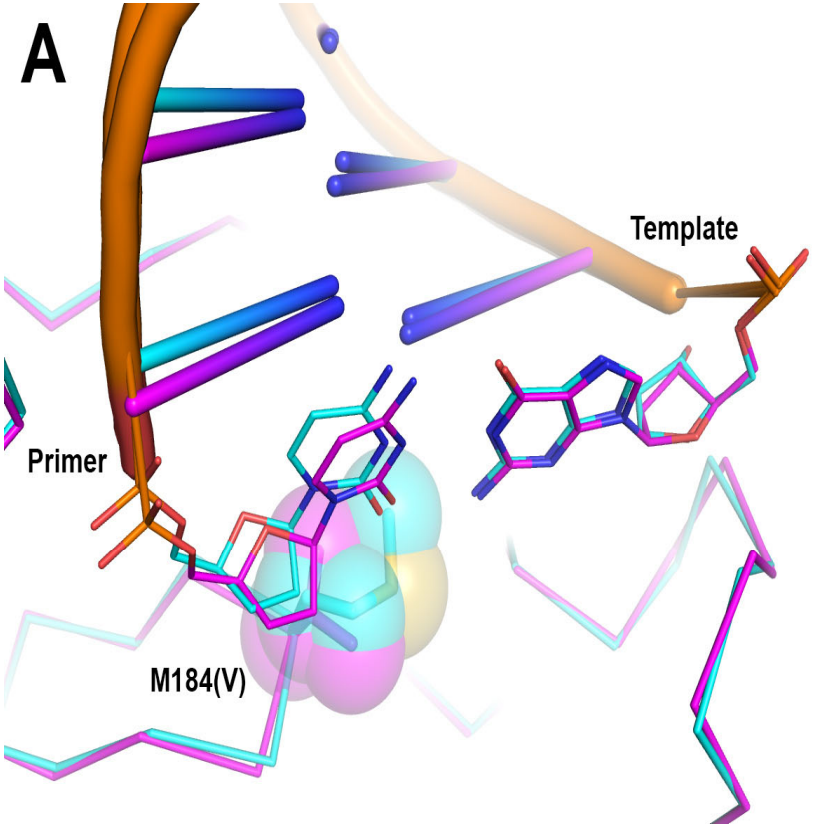
| HIV-1 RT | Nucleotide | $K_d$ (μM) | $k_p$ ($s^{-1}$) | $k_p/K_d$ ($\mu M^{-1} s^{-1}$) | Selectivity factor[a] |
|---|---|---|---|---|---|
| WT | dCTP | 6.3 ± 0.2 | 11.9 ± 0.1 | 1.9 | — |
| WT | (−)-3TC-TP | 0.25 ± 0.01 | 0.068 ± 0.001 | 0.27 | 7.0 |
| WT | (+)-3TC-TP | 0.202 ± 0.004 | 0.26 ± 0.02 | 0.78 | 2.5 |
| WT | (−)-FTC-TP | 0.10 ± 0.01 | 0.100 ± 0.001 | 1.0 | 1.9 |
| WT | (+)-FTC-TP | 0.19 ± 0.02 | 0.24 ± 0.01 | 0.79 | 2.4 |
| M184V | dCTP | 8.9 ± 1.0 | 20.5 ± 0.9 | 2.3 | — |
| M184V | (−)-3TC-TP | 53 ± 4 | 0.050 ± 0.002 | $9.4 \times 10^{-4}$ | 2400 |
| M184V | (+)-3TC-TP | 5.4 ± 1.2 | 0.18 ± 0.01 | $3.3 \times 10^{-2}$ | 69 |
| M184V | (−)-FTC-TP | 28 ± 2 | 0.073 ± 0.001 | $2.6 \times 10^{-3}$ | 880 |
| M184V | (+)-FTC-TP | 1.8 ± 0.3 | 0.13 ± 0.01 | $7.6 \times 10^{-2}$ | 30 |

[a] $(k_p/K_d)_{dCTP}/(k_p/K_d)_{3TC\text{-}TP}$ or $(k_p/K_d)_{dCTP}/(k_p/K_d)_{FTC\text{-}TP}$

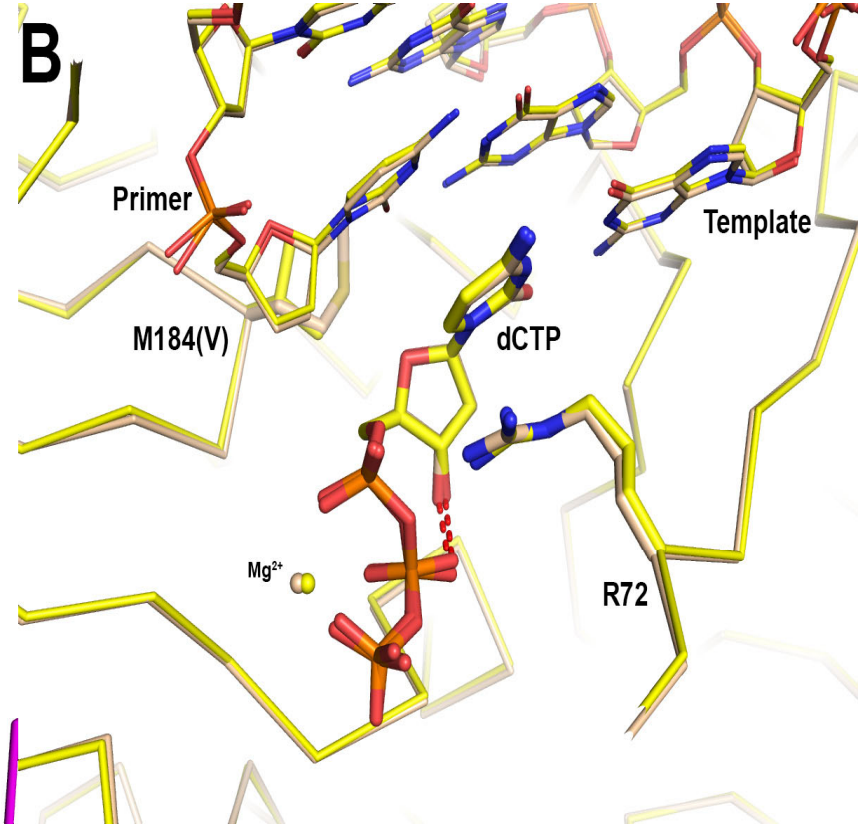
- M184V confers >500 fold resistance to FTC and 3TC in clinicals

[redacted]

# M184 mutation does not affect DNA and dNTP binding




Wt RT-DNA *vs* M184V-DNA




Wt RT-DNA•dCTP *vs* M184V-DNA•dCTP

RT-DNA•(-)-FTC-TP

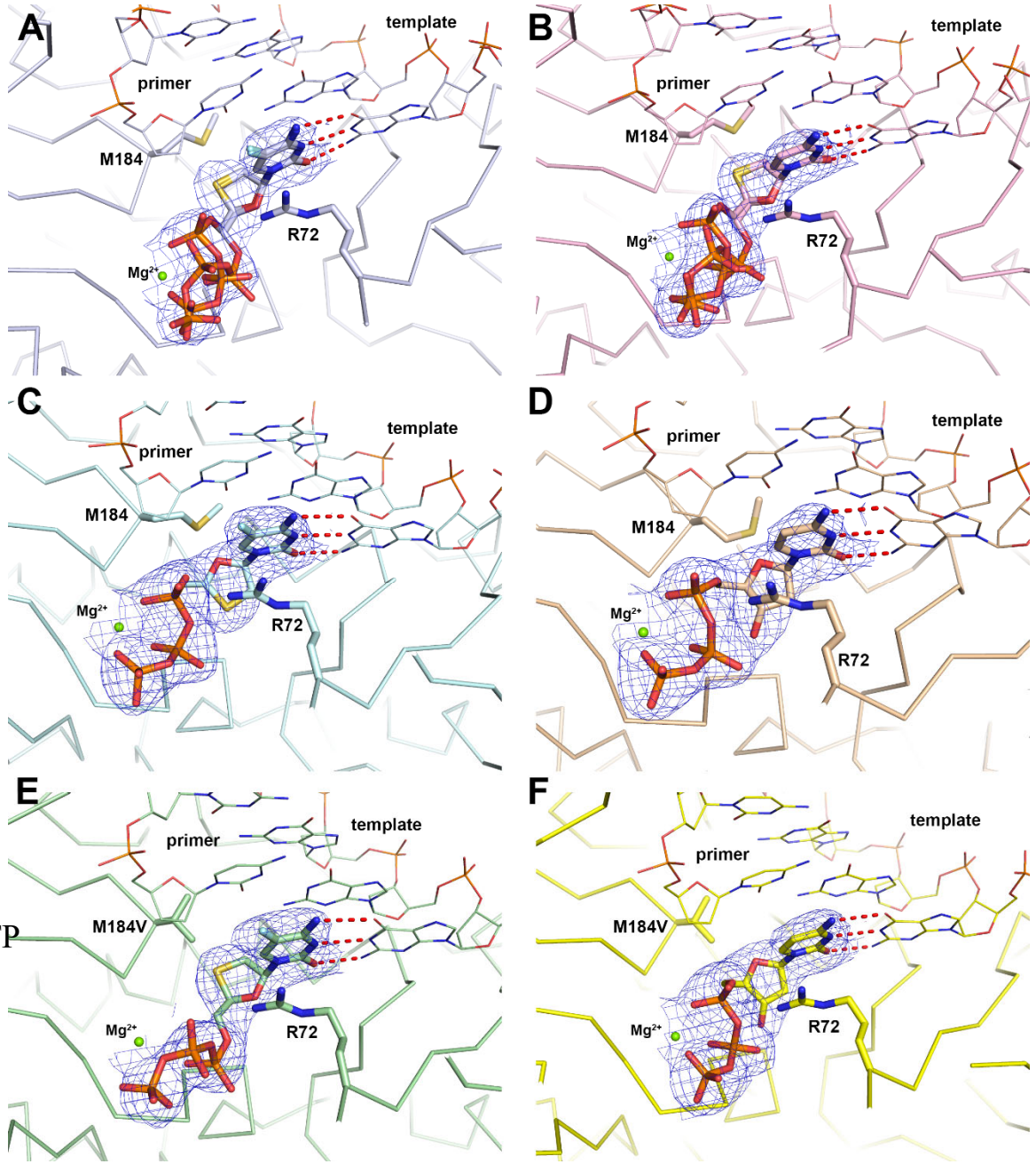
RT-DNA•(-)-3TC-TP

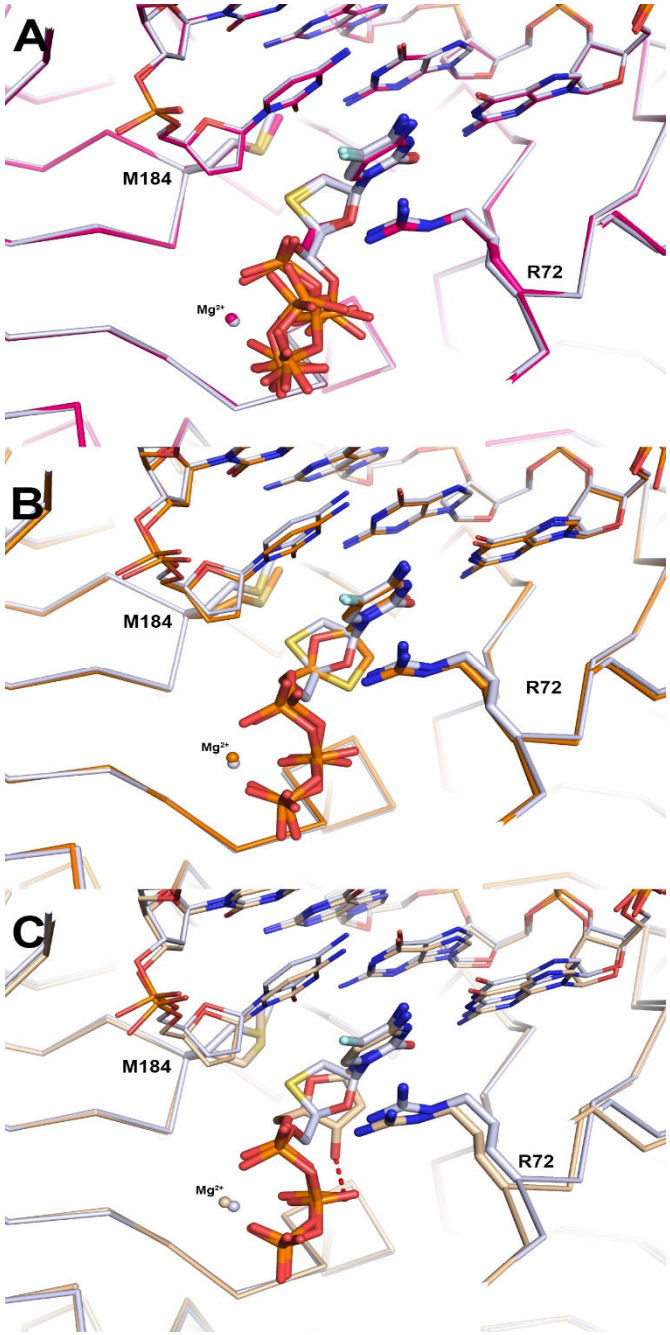
RT-DNA•(+)-FTC-TP

RT-DNA•dCTP

M184V-DNA•(-)-FTC-TP

M184V-DNA•dCTP






Wt RT-DNA•(-)-FTC-TP (light blue)

*vs*

Wt RT-DNA•(-)-3TC-TP (red)

Wt RT-DNA•(-)-FTC-TP (light blue)

*vs*

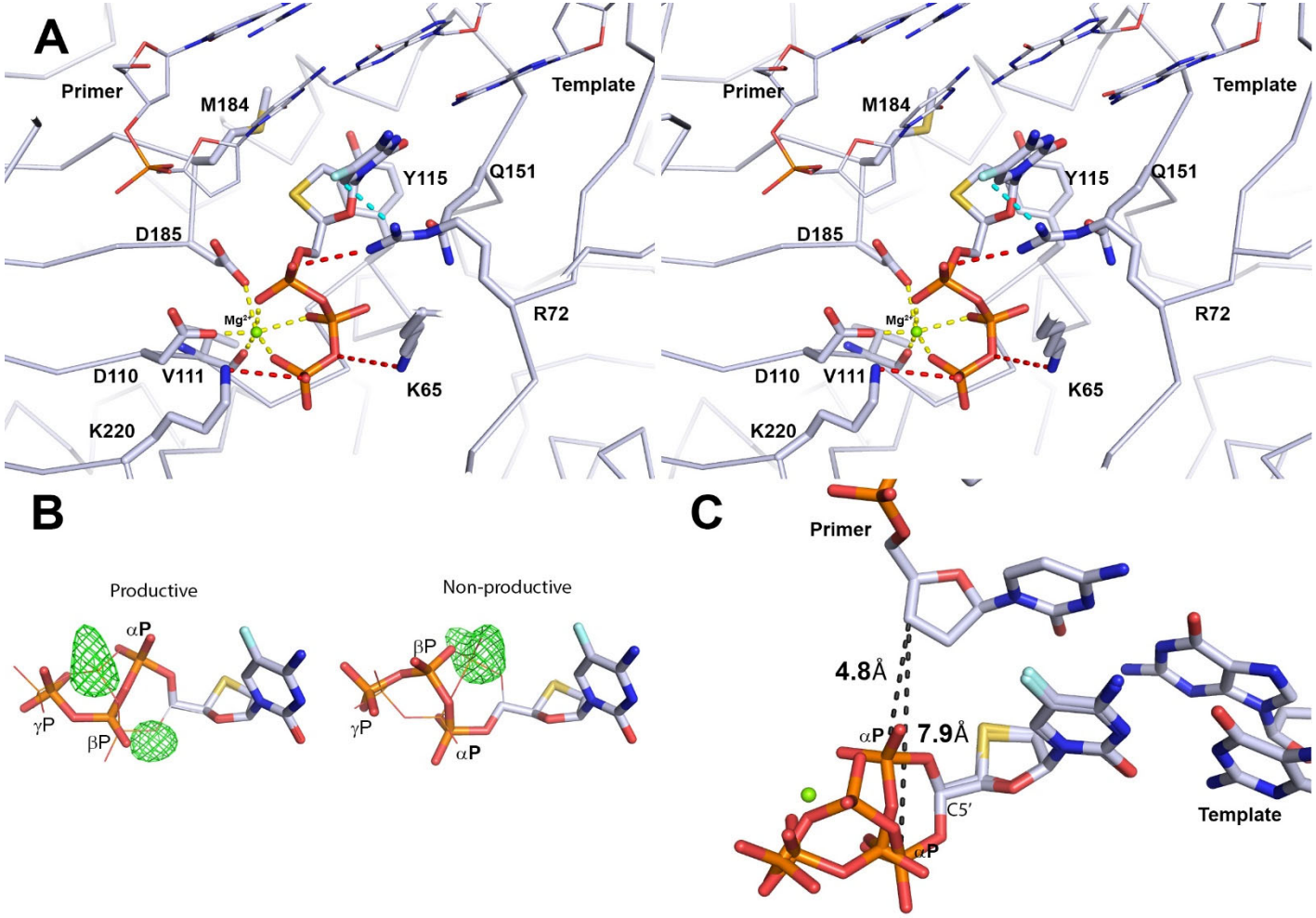
Wt RT-DNA•(+)-FTC-TP (orange)

Wt RT-DNA•(-)-FTC-TP (light blue)

*vs*

Wt RT-DNA•dCTP (beige)

# Ternary crystal structure of HIV-1 RT-DNA•(-)-FTC-TP

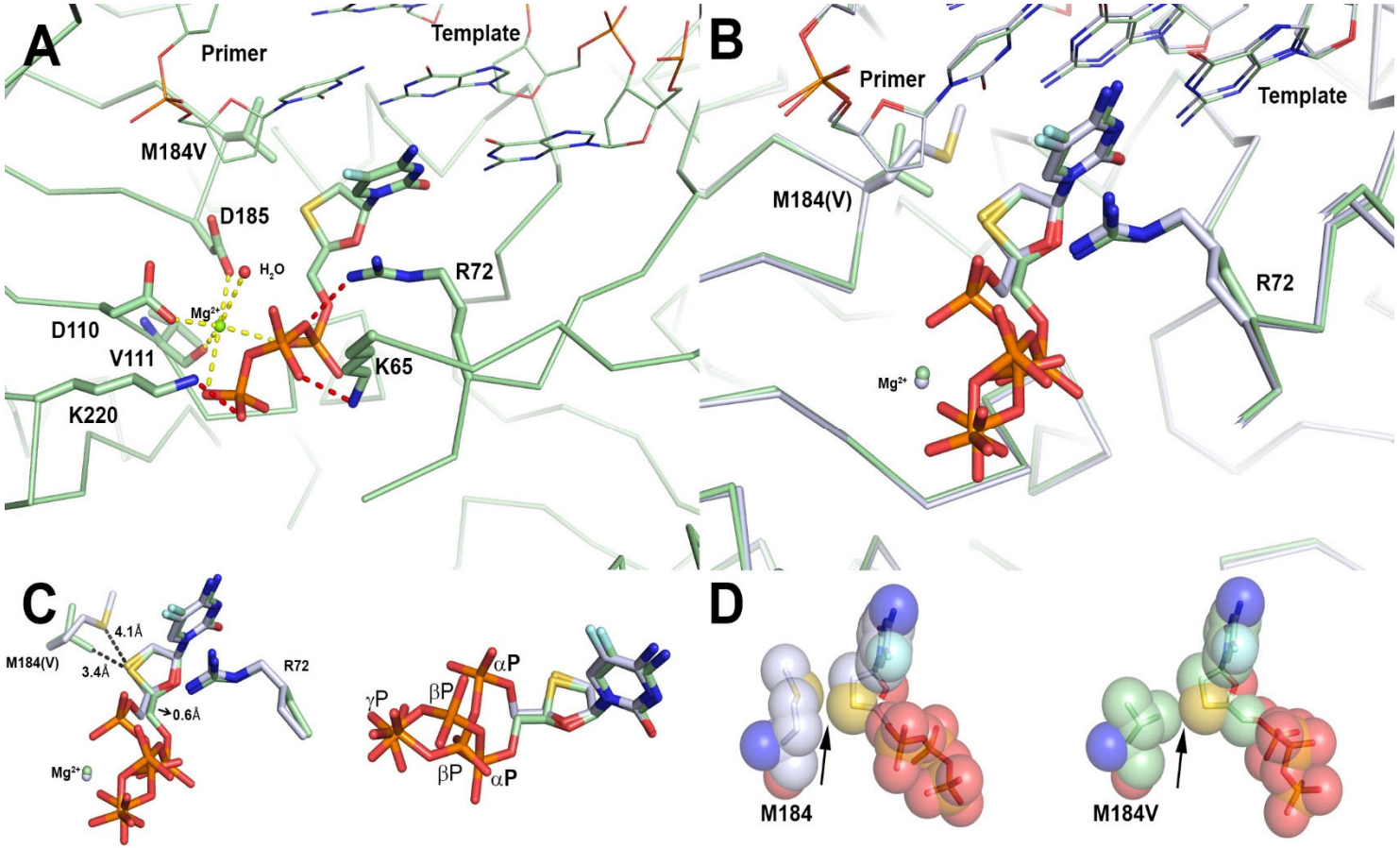



(A) Productive triphosphate binding conformation

(B) and (C) Productive *vs* Non-Productive triphosphate conformation

## M184V-DNA•(-)-FTC-TP

## Wt RT-DNA•(-)-FTC-TP *vs* M184V-DNA•(-)-FTC-TP




(-)-FTC-TP conformations

M184V clashes with sulfur in (-)-FTC-TP

# Conclusions:

- (-)-FTC-TP and (-)-3TC-TP have higher binding affinities for wild-type HIV-1 RT but slower incorporation rates than dCTP.
- The higher binding affinities of the *L*-analogs are due to the sulfur atom in their oxathiolane while their lower incorporation rates are due to their two different triphosphate binding conformations.
- Relative to dCTP, M184V displays >200-fold and >9-fold lower affinities towards the *L*-nucleotides and the *D*-isomers, respectively.
- The M184V mutation repositions the oxathiolane of the *L*-nucleotides and shifts their triphosphate into a non-productive conformation

Hung, M., Tokarsky, E.J., Lagpacan, L., Zhang, L., Suo, Z., & Lansdon, E.B. (2019) *Nature Commun Biol.* 2:469.

# Acknowledgments


## Contributors

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Prof. Feng Zhang (Broad Institute of Harvard/MIT)

## Financial Support

Startup fund from FSU
Gilead Sciences, Inc.
NIH (R01GM122093, R01GM124177, R01ES028271, R01CA211175, and R01GM089846)
NSF (MCB-1716168)