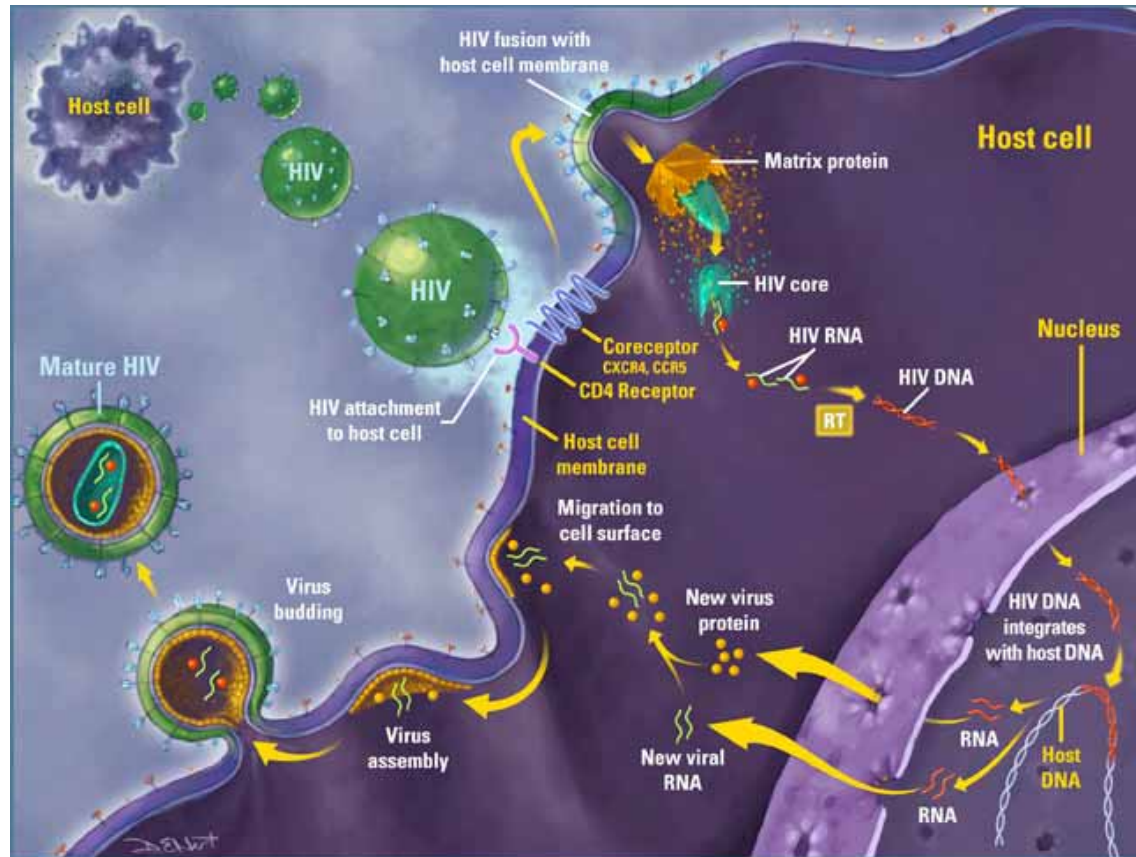


# HIV -1 Reverse Transcriptase

Sravanti Vaidya



# HIV Life Cycle



HIV Web Study ([www.HIVwebstudy.org](http://www.HIVwebstudy.org))

Supported by HRSA

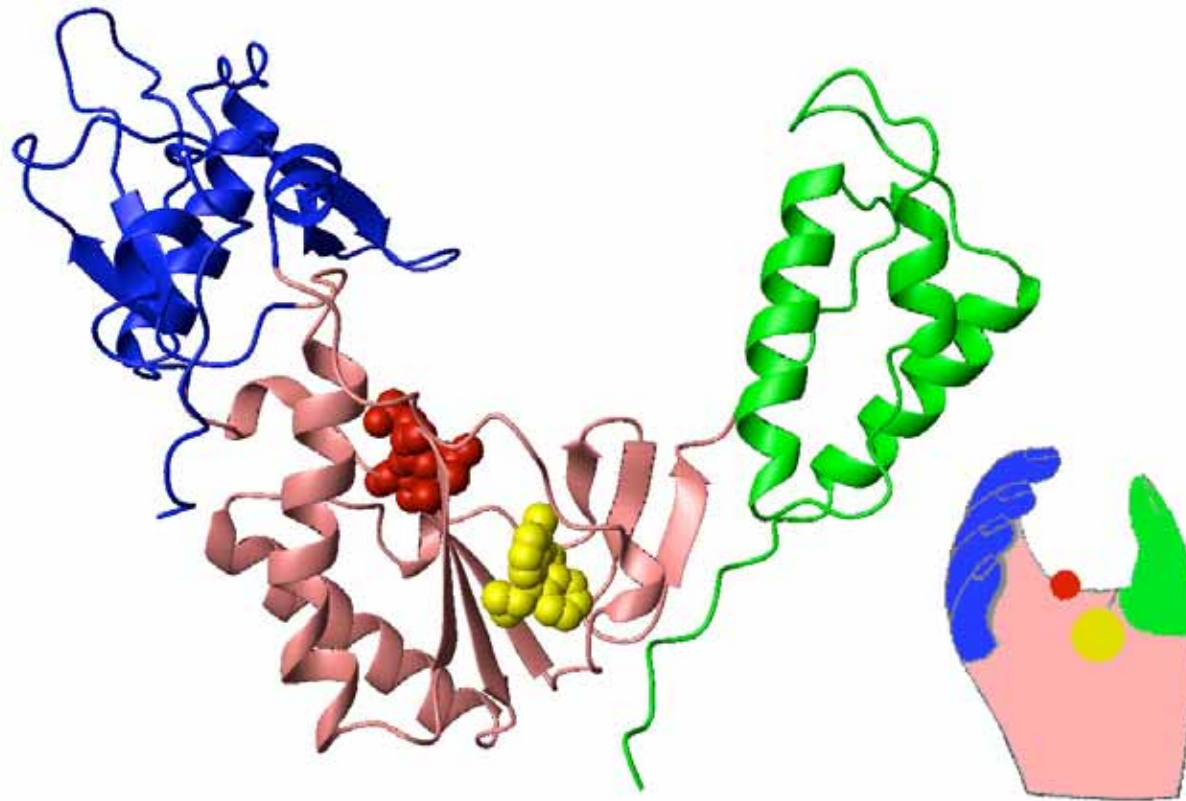
## Structure of RT

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- It's a heterodimer with 2 chains A,B
- 
- a 51-kD subunit (p51) of -440 amino acids – DNA polymerase activity
- a 66-kD subunit (p66) of 560 amino acids –DNA polymerase domain and RNase activity
  
- The polymerase domain is divided into four sub domains:
  - the fingers –residues 1-85, 118-155
  - the palm - residues 86-117, 156-237
  - the thumb -residues 238-318
  - the connection -residues 319-426
  - analogous to the human right hand.

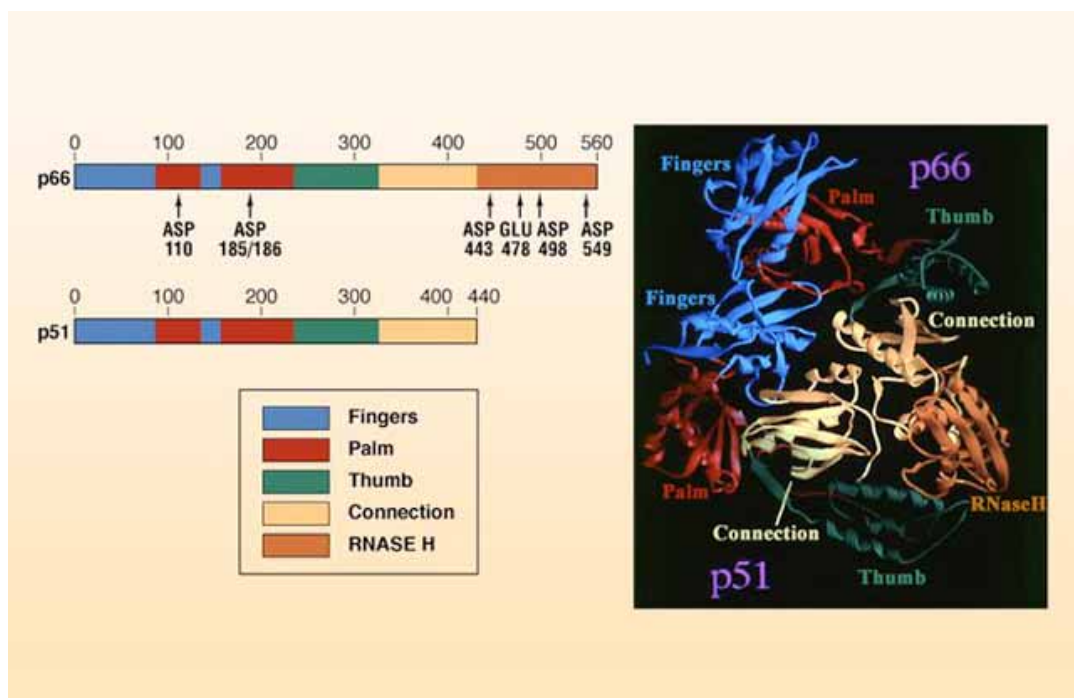
## Subdomains of the p66 subunit

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## p51 subunit

- The p51 subunit has only the polymerase domain
- same fingers, thumb, palm and connection sub domains, however, their spatial arrangement differs markedly to those of the p66 subunit.



## Asymmetric heterodimer

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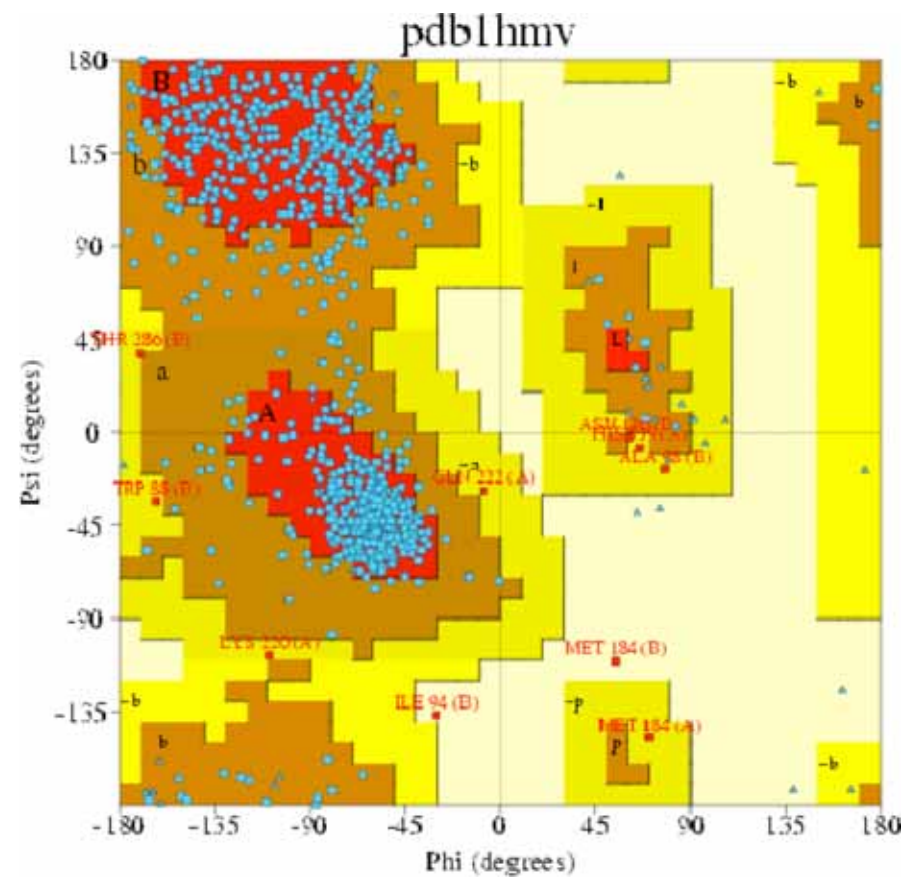
- A fascinating feature of the HIV-1 RT heterodimer is the structural asymmetry
- despite the fact that they are products of the same gene and exhibit identical amino acid sequences for the first 440 residues.
- The polymerase domain of p66 folds into an open extended structure containing a large active-site cleft while that of p51 is closed and compact (5).

## Crystal characteristics

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- Resolution: 3.20Å  
R-factor: 0.254  
R-free: 0.297
- Chain A:
  - Number of Alpha -**15** Content of Alpha-**30.00**
  - Number of Beta- **23** Content of Beta-**19.11**
- Chain B:
  - Number of Alpha -13 Content of Alpha -29.32
  - Number of Beta -15 Content of Beta -15.23

Glycine residues -54  
 Proline residues -66





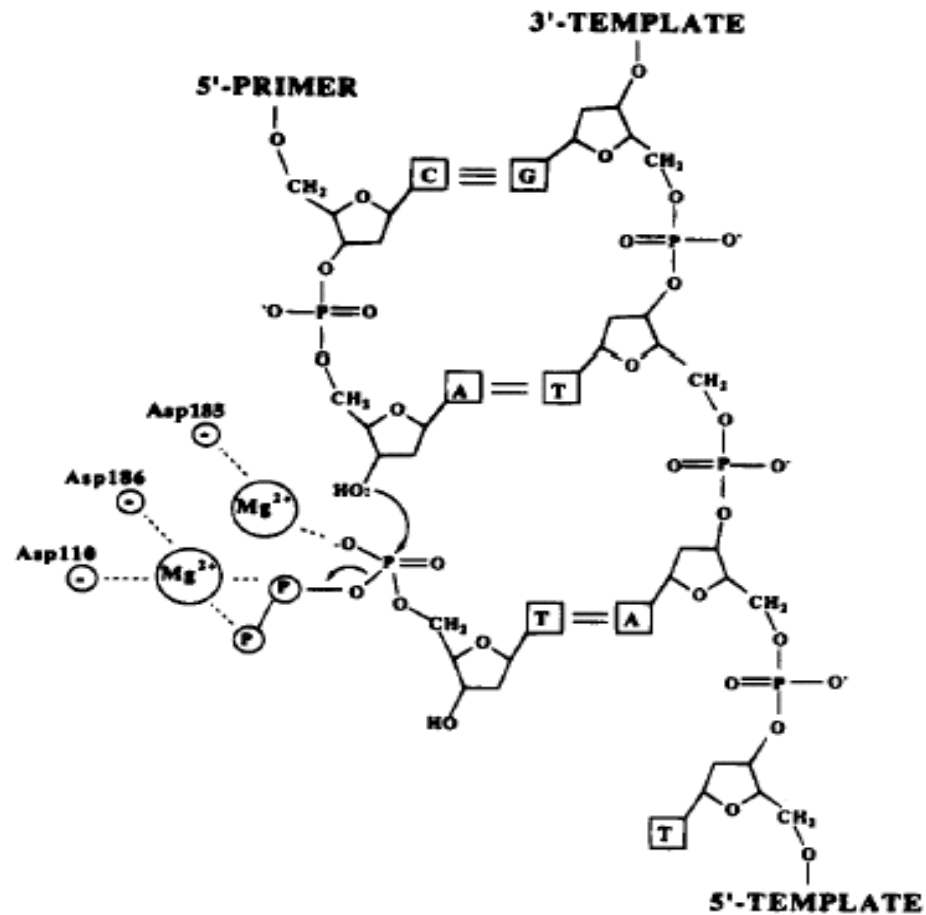
## Two enzymes in One !!!!

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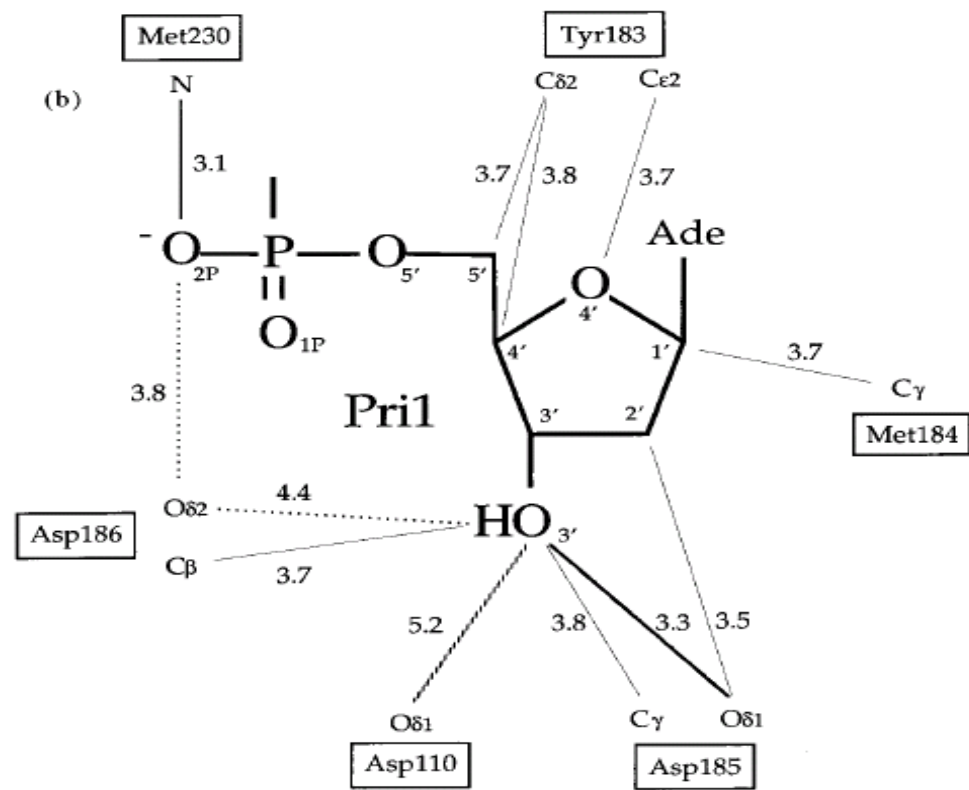
- Functions
  - 5'-->3' RNA directed DNA polymerization
    - -synthesizes DNA on the RNA template
  - RNase -degrades RNA in the RNA: DNA hybrid
  - 5'-->3' DNA directed DNA polymerization
    - synthesizes DNA on the DNA template

- 
- In addition to requiring DNA elongation and RNA degradation activities of the enzyme, complete reverse transcription of genome requires two initiation events one using
  - tRNA Lys-3 as a primer for minus-strand DNA synthesis
  - one using RNase H-resistant polypurine tract RNA as a primer for positive-strand DNA synthesis (4)

# Active sites



# YMDD interactions



## Mode of action of RT inhibitors

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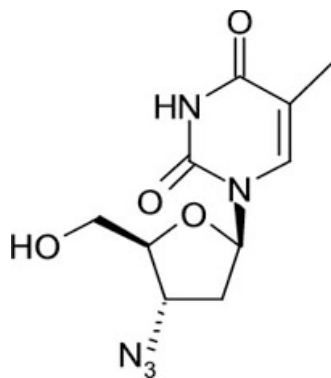
- The inhibitors can act at various steps of the life cycle and inhibit the activity.
  
- The RT inhibitors
- Protease inhibitors
- Entry inhibitors
  
- RT inhibitors:
  - NRTI- Nucleoside/Nucleotide reverse transcriptase inhibitor
  - NNRTI- Non Nucleoside reverse transcriptase inhibitor

# UMassAmherst

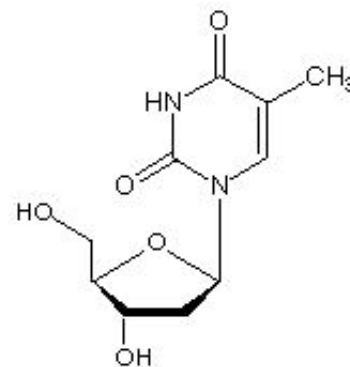
## Nucleoside analogues

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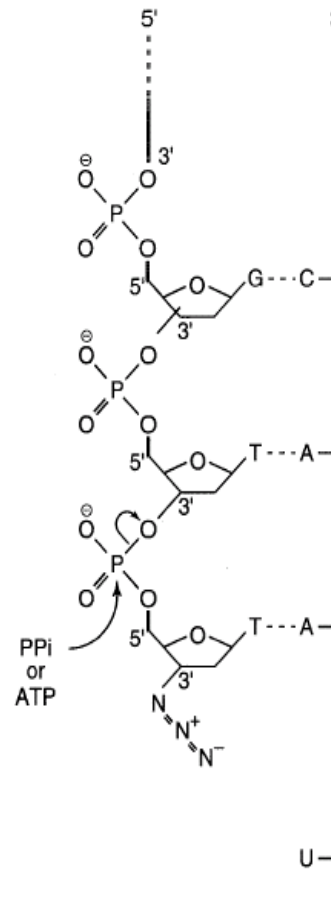
3'-Azido-3'-deoxythymidine



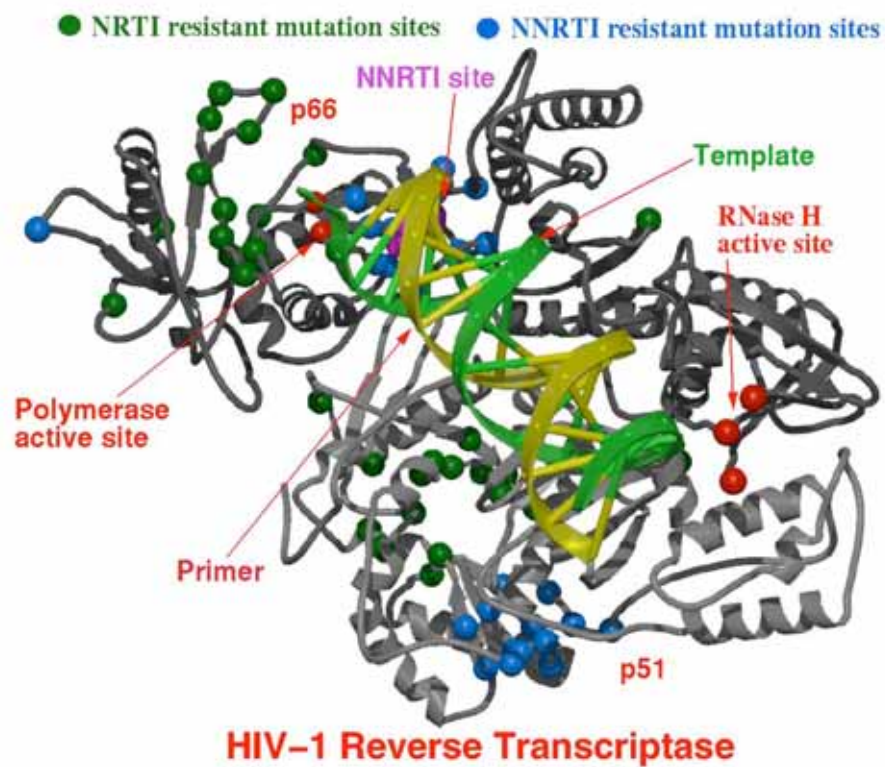
Thymidine



# Mode of action



# RT Inhibitors



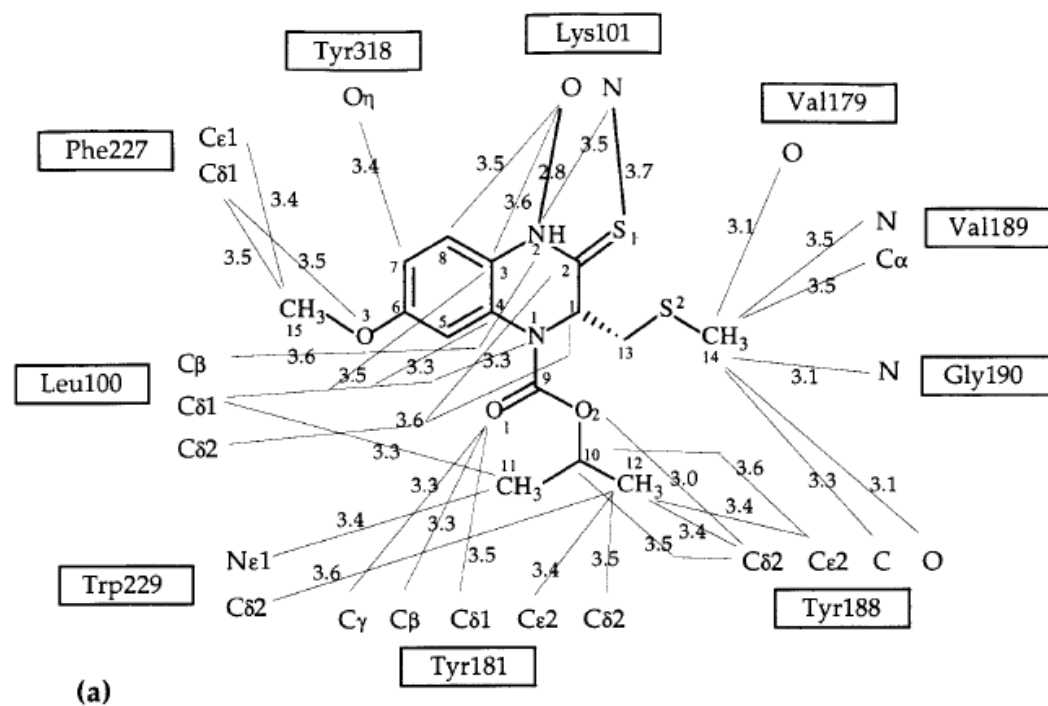


## NNRTI

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- NNRTIs are noncompetitive inhibitors,
- bind to a hydrophobic pocket in RT near the polymerase active site and prevent RT from carrying out the polymerization reaction.
- NNRTIs do not interfere with substrate binding

# Interactions of HBV



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- **References:**

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- 2) Ding, J., Das, K., Hsiou, Y., Sarafianos, S., Clark, A., Molina, J., Tantillo, C., Hughes, S., Arnold, E., (1998). Structure and Functional Implications of the Polymerase Active Site Region in a Complex of HIV-1 RT with a Double-stranded DNA Template-primer and Antibody Fab Fragment at 2.8 Ao Resolution. *J. Mol. Biol.* **284**, 1095-1111.
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