

# A survey of structure and dynamics in HIV-1 Reverse Transcriptase

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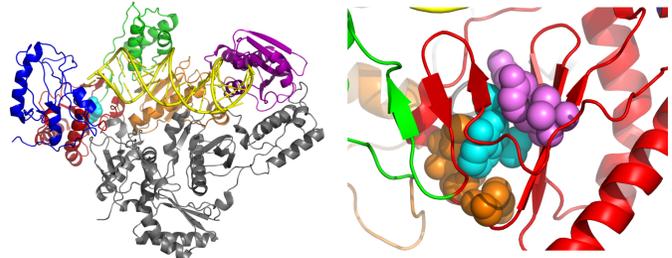
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## Abstract

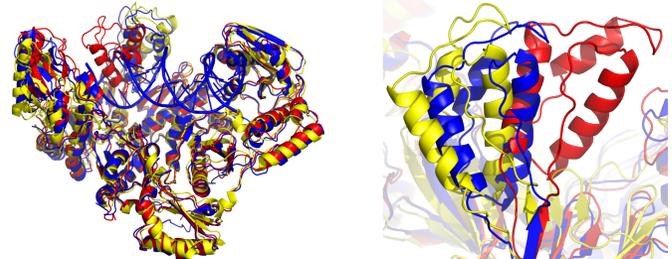
HIV-1 reverse transcriptase (RT) is a critical drug target for HIV treatment, and understanding the exact mechanisms of its function and inhibition would significantly accelerate the development of new anti-HIV drugs. Structural information on reverse transcriptase alone has proven to be insufficient to explain the mechanism of inhibition and drug resistance of non-nucleoside reverse transcriptase inhibitors. Elastic network modeling provides a technique to rapidly probe and compare protein dynamics. Combining elastic network modeling with hierarchical clusters of both structural and dynamic data reveals a wealth of novel information. Here we present an extensive survey of the dynamics of reverse transcriptase bound to a variety of ligands with a number of mutations, revealing a novel mechanism for drug resistance to non-nucleoside reverse transcriptase inhibitors, where hydrophobic core mutations subtly shift the position of the thumb subdomain, restoring active-state motion to multiple functionally significant regions of HIV-1 RT. This model arises out of a combination of structural and dynamic information, rather than exclusively from one or the other.

## Structure of HIV-1 RT



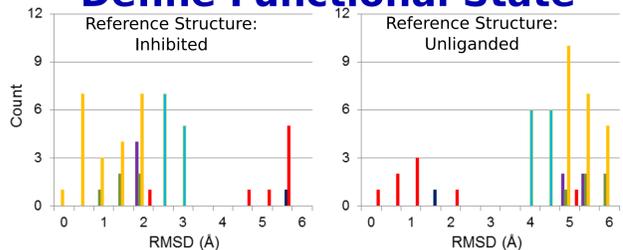
- p66 subunit (colored)
  - Fingers subdomain (blue)
  - Palm subdomain (red)
  - Thumb subdomain (green)
  - Connection domain (orange)
  - RNase H domain (purple)
- p51 subunit (grey)
- Drug binding pocket
- NNRTI (cyan spheres)
- Hydrophobic core residues (purple spheres)
- Entry blocker residues (orange spheres)

## States of HIV-1 RT



- Unliganded HIV-1 RT (red)
- DNA-bound HIV-1 RT (blue)
- NNRTI-bound HIV-1 RT (yellow)
- Thumb rotates based on ligand
- NNRTIs cause hyper-extension

## Structural Comparison Cannot Define Functional State

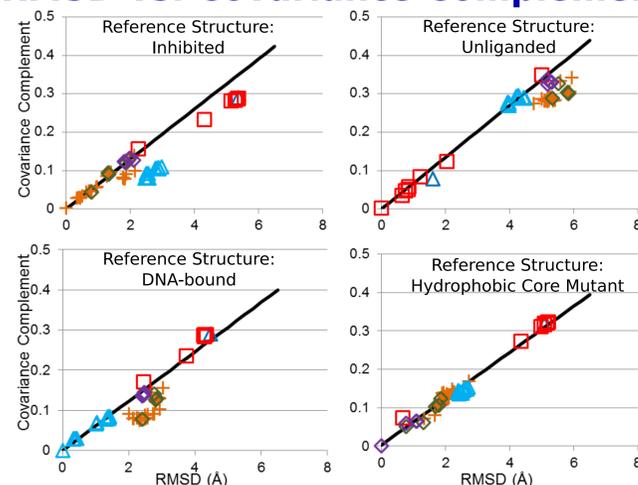


- Unliganded (red)
- DNA-bound (light blue)
- RNA-bound (dark blue)
- NNRTI-bound wild type or susceptible (yellow)
- NNRTI-bound Hydrophobic core mutant (purple)
- NNRTI-bound entry blocker mutant (green)

## Elastic Network Models

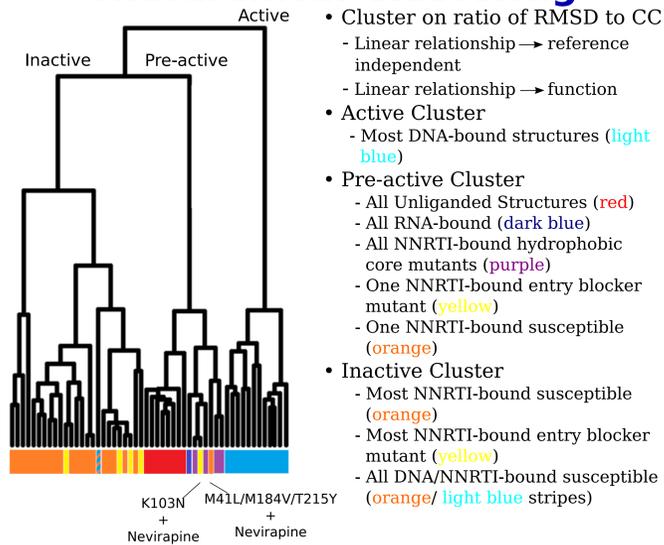
- All-atom Molecular Dynamics (MD) too slow
  - Coarse-grained model, Cα resolution, fast
  - "Beads on springs"
  - Single harmonic potential:  $U_{ij} = k(r_{ij}) (|r_{ij}| - |r_{ij}^0|)^2$
  - Covariance Complement
  - Compares two ENM results
- $$CC_{A,B} = \left[ \frac{\sum_i (\lambda_i^A + \lambda_i^B) - \sum_i \sum_j \sqrt{\lambda_i^A \lambda_j^B} (\vec{v}_i^A \cdot \vec{v}_j^B)^2}{\sum_i (\lambda_i^A + \lambda_i^B)} \right]^{1/2}$$
- λ is the inverse of the eigenvalue  
 -v is the eigenvector  
 -Quantify similarity of modes  
 -Scales [0:1]  
 -1 is completely orthogonal  
 -0 is complete overlap  
 Hess, Phys Rev E (2000), 62, 8438-48

## RMSD vs. Covariance Complement



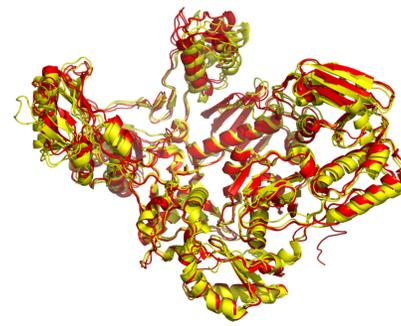
- Linear relationship between like functional states
- DNA-bound (light blue triangle)
- RNA-bound (dark blue triangle)
- NNRTI-bound wild type or susceptible (orange cross)
- NNRTI-bound hydrophobic core mutant (purple diamond)
- NNRTI-bound entry blocker mutant (green diamond)
- Unliganded (red square)

## Hierarchical Clustering



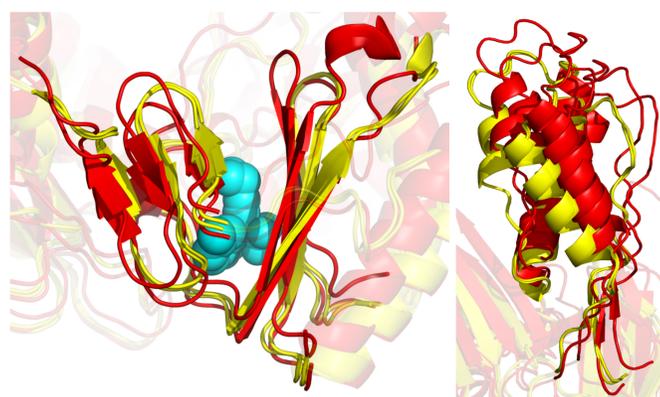
- Cluster on ratio of RMSD to CC
- Linear relationship → reference independent
- Linear relationship → function
- Active Cluster
  - Most DNA-bound structures (light blue)
- Pre-active Cluster
  - All Unliganded Structures (red)
  - All RNA-bound (dark blue)
  - All NNRTI-bound hydrophobic core mutants (purple)
  - One NNRTI-bound entry blocker mutant (yellow)
  - One NNRTI-bound susceptible (orange)
- Inactive Cluster
  - Most NNRTI-bound susceptible (orange)
  - Most NNRTI-bound entry blocker mutant (yellow)
  - All DNA/NNRTI-bound susceptible (orange/light blue stripes)

## Structural Differences are Subtle



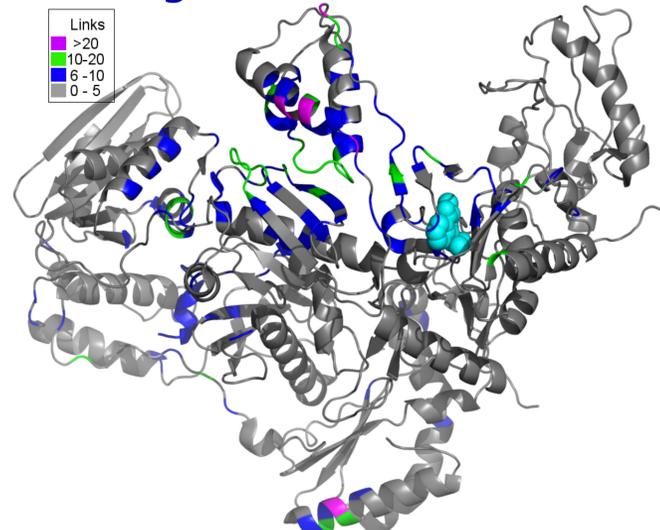
- Pre-active structures (red)
- Inactive structures (yellow)
- Structures all in hyperextended conformation
- Fingers, thumb, and RNase H domains all subtly different
- Other structures largely unchanged

## Deformation of Drug Binding Pocket Alters Thumb Position



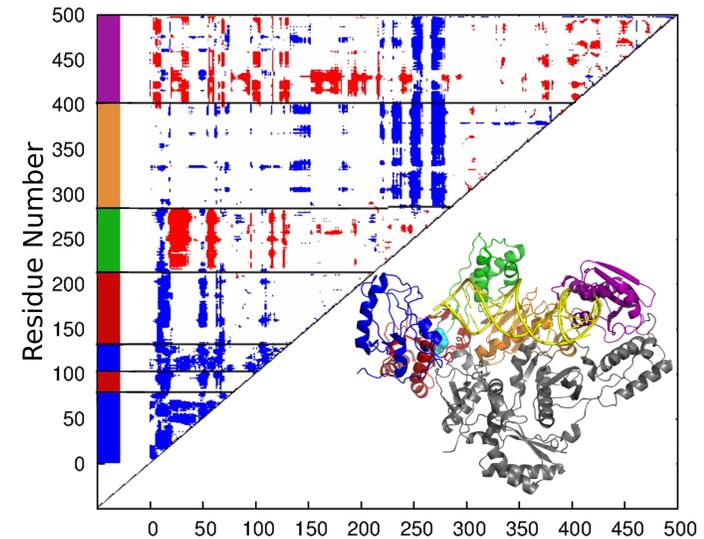
- Pre-active structures (red)
- Inactive structures (yellow)
- NNRTI (cyan)
- Subtle deformation of binding pocket
- Rotation of β-12-13-14
- Pre-active thumb subdomain lifts away from connection domain
- Inactive thumb subdomain rests directly against connection subdomain

## Changes in Residue Contacts



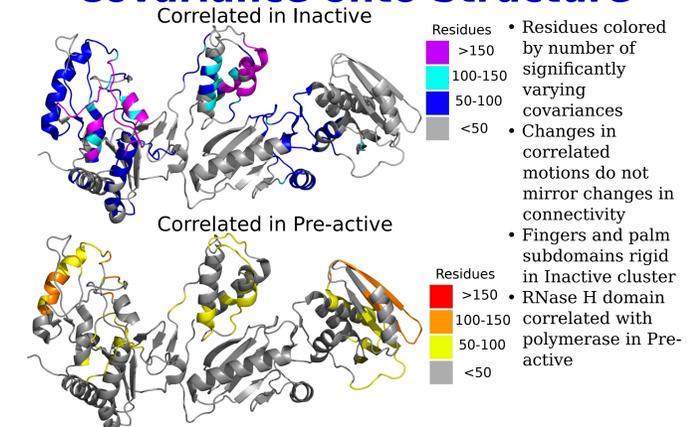
- ENM insensitive to small changes in number of residue contacts
- Connectivity changes strongest in thumb subdomain
- Subtle structural changes lead to large connectivity changes
- Large connectivity changes lead to dynamics changes
- NNRTI (cyan)

## Difference in Correlated Motion



- Plot of significant differences in covariance between inactive and pre-active cluster
- Motion correlated in Inactive only (blue)
- Motion correlated in Pre-active only (red)
- Fingers subdomain (blue)
- Palm subdomain (red)
- Thumb subdomain (green)
- Connection domain (orange)
- RNase H domain (purple)

## Mapping Changes in Covariance onto Structure



- Residues colored by number of significantly varying covariances
- Changes in correlated motions do not mirror changes in connectivity
- Fingers and palm subdomains rigid in Inactive cluster
- RNase H domain correlated with polymerase in Pre-active
- Dynamics and structure both requires to characterize function
- Structures with similar function show a linear relationship between structure and dynamics
- MD not bioinformatics tool
- ENMs can be bioinformatics tools
- Subtle changes in structure can lead to larger changes in dynamics
- Hydrophobic core mutations cause a change in structure/dynamics
- Rotation of thumb
- Restores unliganded motions
- Small changes in structure can lead to distant changes in dynamics

Work done in LOOS (Lightweight Object Oriented Structure analysis library), an open source C++ library designed and maintained by the Grossfield lab. LOOS provides a concise, adaptable framework for designing analysis tools that interfaces with native file formats of most simulation packages.  
<http://loos.sourceforge.net>

