Molecular Dynamics of HIV-1 Reverse Transcriptase

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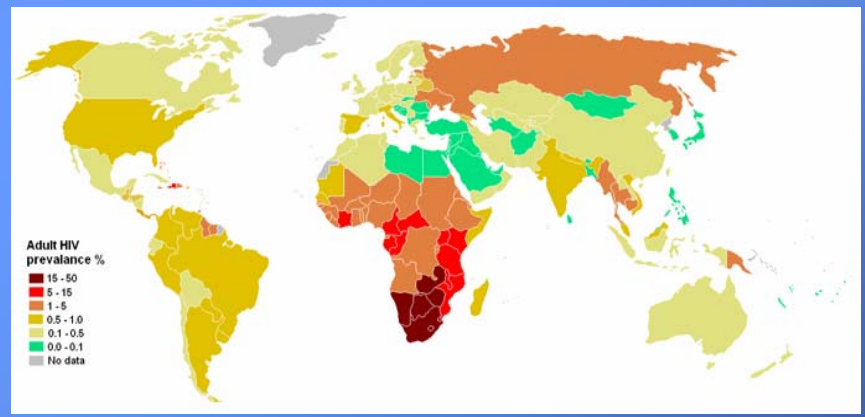
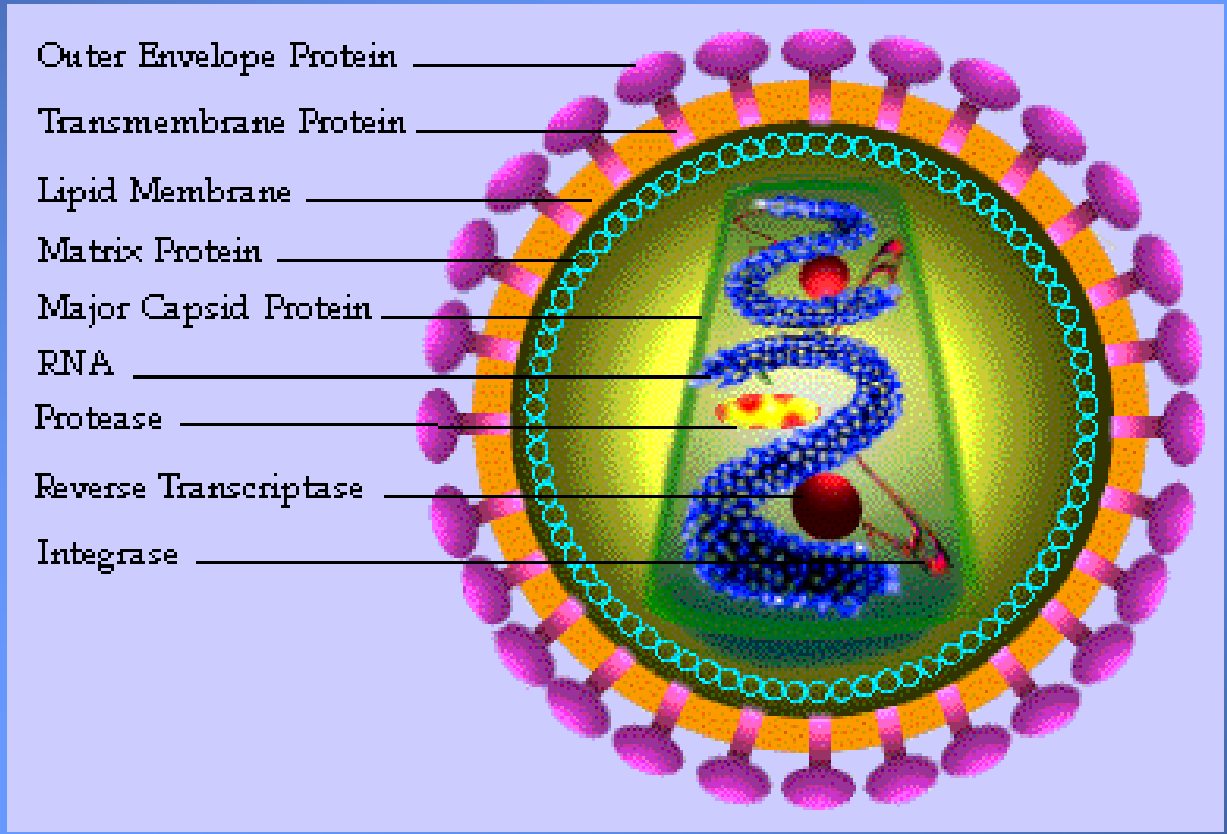
Outline

- HIV-1 Reverse Transcriptase (RT)
  - Infection is a pandemic
  - 25 million since 1981
  - 33-46 million living
- Molecular Dynamics
- Results
- Conclusions and Future Studies



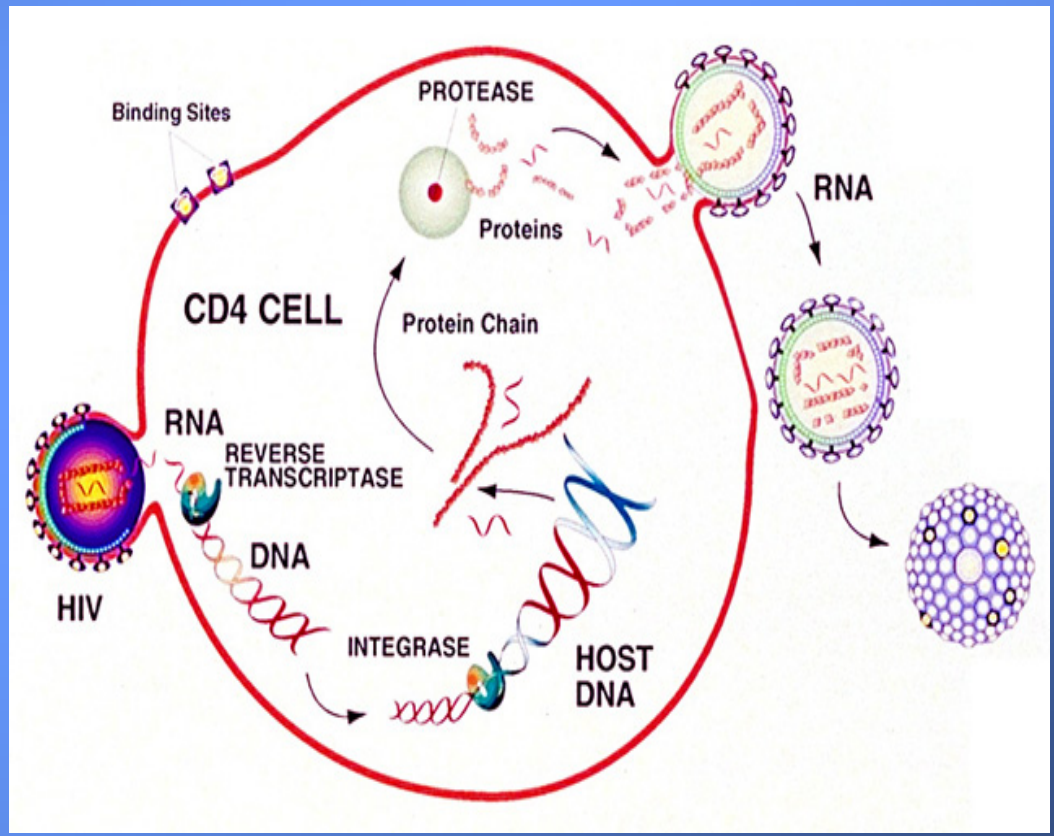
HIV Reverse Transcriptase



Replication of the HIV virus

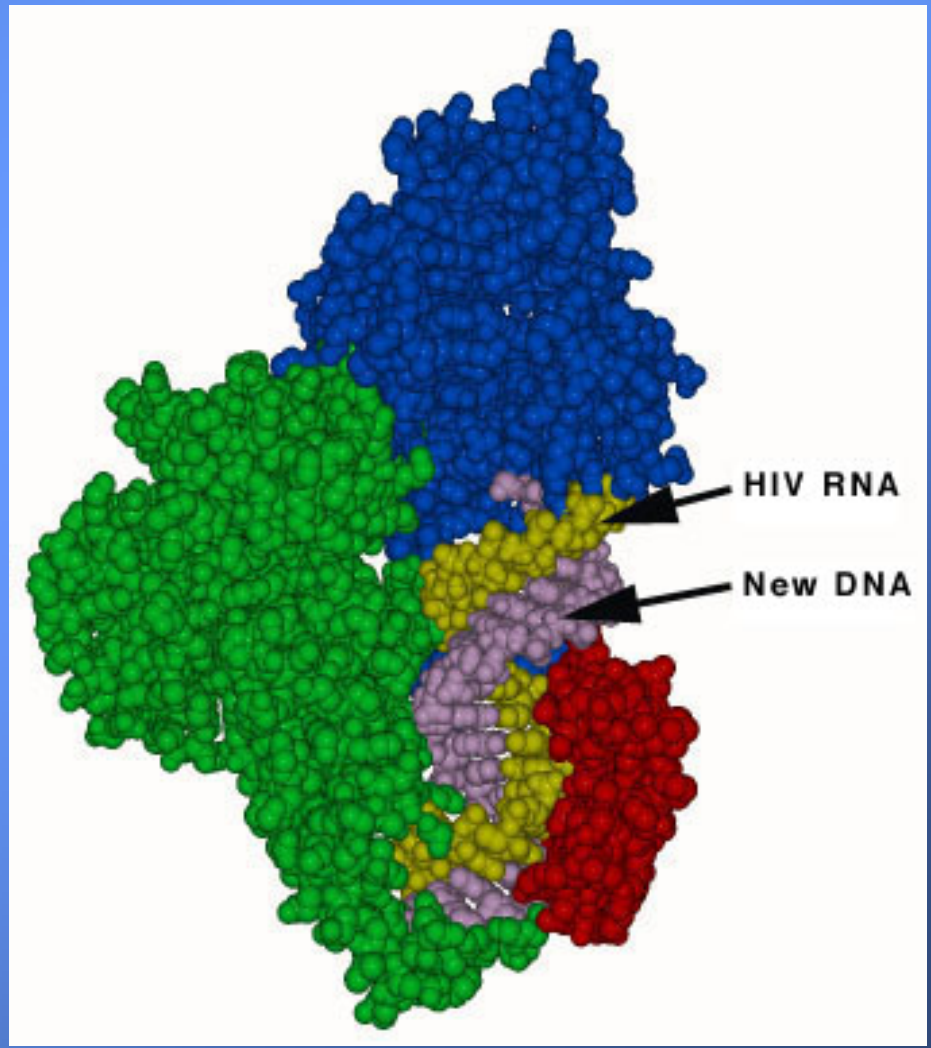
- Binding to specific protein on cell
- Fusion & entry into cell
- Reverse Transcriptase Converts genetic

  RNA → DNA
- Integrase integrates viral DNA into host DNA
- DNA → mRNA → viral protein
- HIV protease cleaves viral proteins, which are assembled on cell surface
- Mature virus released



Complex of HIV -1 RT with DNA

- Hetero-dimer
  - P66 (blue + red)
    - Ribonuclease (red): digest RNA once DNA copy made
    - DNA Polymerase (blue): copies RNA template
  - P51 (green)
    - DNA Polymerase
    - No Ribonuclease domain
- Identical in primary sequence except for ribonuclease subunit

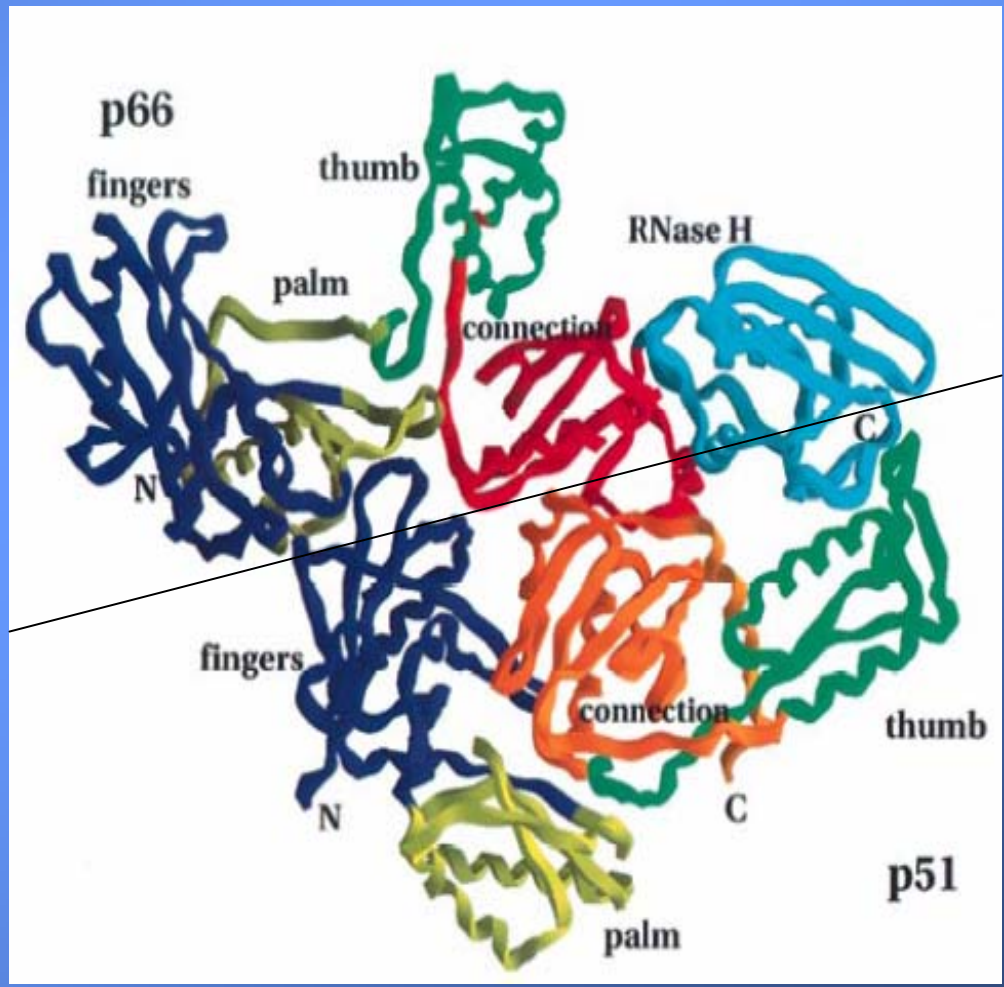


Complex of HIV-1 RT with Nonnucleoside Inhibitor (Nevirapine)

- Polymerase Domain

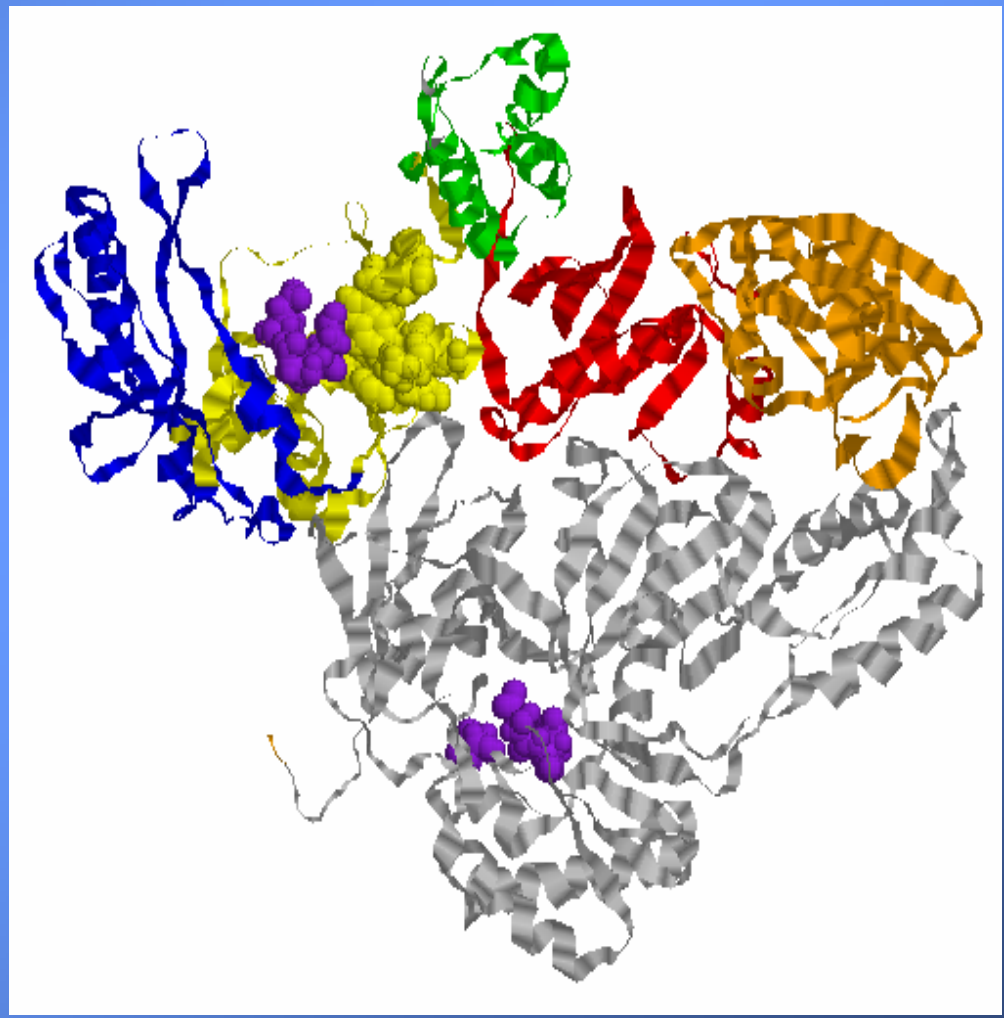
  "Right Hand"

  - Subdomains
    - Fingers
    - Palm
    - Thumb (flexible)
    - Connection
  - Subdomains have identical primary structure but differ in tertiary structure



Complex of HIV-1 RT with dsDNA

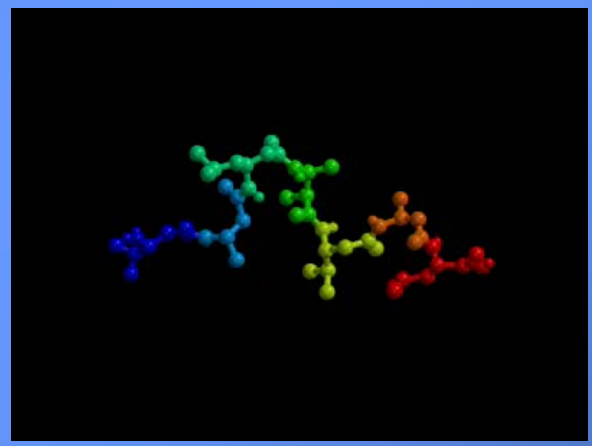
- P66
  - Open hand with binding cleft between thumb & fingers for DNA
  - Functional catalytic active site (palm): 3 Asp residues
  - Site for non-nucleoside inhibitors (hydrophobic)
- P51 (rigid)
  - No DNA-binding cleft
  - No functional catalytic active site
  - No inhibitor binding site



Motivation

- Understanding the conformation flexibility of HIV-1 RT is essential in
  - Controlling mechanism of polymerase
  - Binding of inhibitors
  - Developing more efficient drugs

Molecular Dynamics



- Theoretical studies of biological molecules permit the study of relationship between structure, function and dynamics on atomic level.
- MD calculates “real” dynamics of the system, from which time average properties can be calculated.

Inputs:

1) coordinate of atoms, pdb file from protein data bank.
2) potential energy function, determine atomic interaction forces.

MD cont'd…

- Force Fields:
  - Four component picture of the intra and inter molecular forces with in the system
  - Provides a function to describe energy change
  - As opposed to quantum mechanical methods,
    - Ignore the electronic motions
    - calculations are not time consuming
  - Transferability

MD cont'd …

Newton's laws F= Ma

$$\frac{d^2 r_i}{dt^2} = F_i(r_1, r_2, \cdots, r_n) \Big/ m_i$$

$$F_i(r_1, r_2, \cdots, r_n) = -\nabla V(r_1, r_2, \cdots, r_n) \qquad i = 1, 2, \cdots, N$$

$$V_i(\vec{r}) = V_i(\vec{r}_1, \vec{r}_2, \vec{r}_3, \ldots, \vec{r}_N)$$

$$= \sum_{bonds} \frac{1}{2} K_b (b - b_0)^2 + \sum_{angles} \frac{1}{2} K_q (q - q_0)^2 + \sum_{improper} \frac{1}{2} K_x (x - x_0)^2 +$$

$$\sum_{dihedral} K_j \left[1 + \cos(n_j - d)\right] + \sum_{ij} \left[\frac{C_{12}}{r_{ij}^{12}} - \frac{C_6}{r_{ij}^6} - \frac{q_i q_j}{4\pi\varepsilon_0 \varepsilon_g r_{ij}} \right]$$

MD cont'd …

Potential Energy

- Bonded forces (bonds angles, dihedrals, impropers)
- Non-bonded forces (coulomb, Van der Waals)

$$V_i(\vec{r}_1, \vec{r}_2, \vec{r}_3, \ldots, \vec{r}_N)$$

$$= \sum_{bonds} \frac{1}{2} K_b (b - b_0)^2 + \sum_{angles} \frac{1}{2} K_q (q - q_0)^2 + \sum_{improper} \frac{1}{2} K_x (x - x_0)^2 +$$

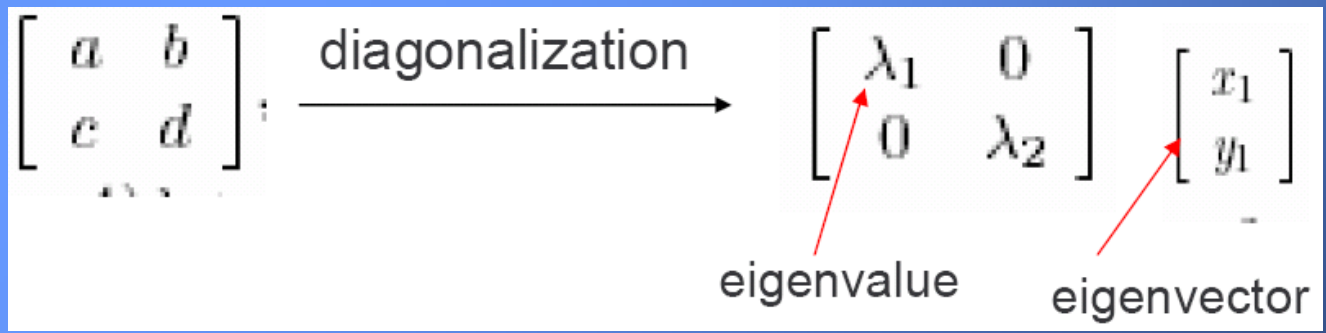
$$\sum_{dihedral} K_j \left[1 + \cos(n_j - d) \right] + \sum_{ij} \left[\frac{C_{12}}{r_{ij}^{12}} - \frac{C_6}{r_{ij}^6} - \frac{q_i q_j}{4\pi\varepsilon_0 \varepsilon_g r_{ij}} \right]$$

MD cont'd …

- Covariance Matrix $\langle x_i \rangle$ $x_i - \langle x_i \rangle$
  - Build covariance matrix of positional fluctuations.

$$COV(x_i x_j) = \langle (x_i - \langle x_i \rangle)(x_j - \langle x_j \rangle) \rangle$$

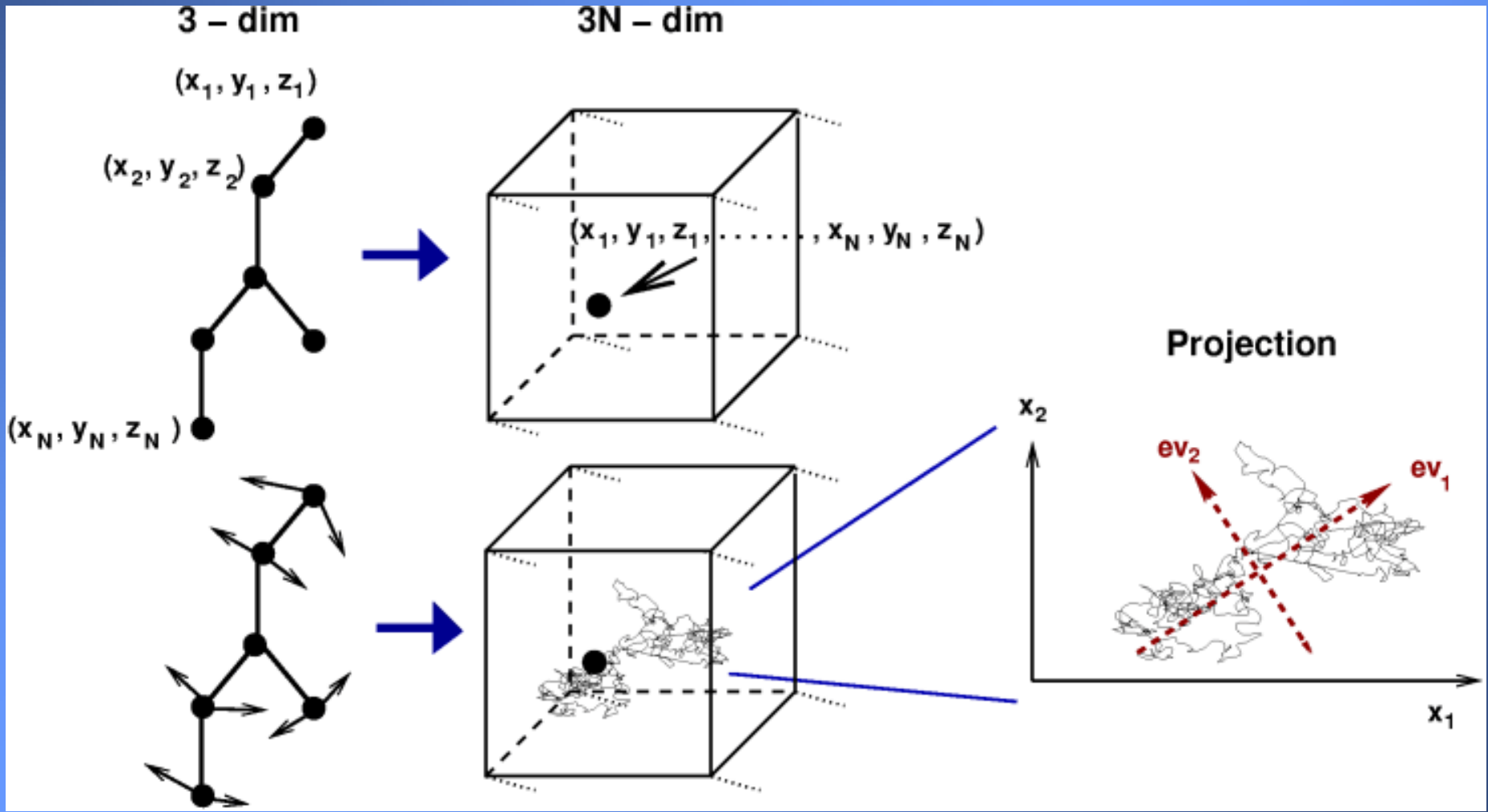
  - Diagonalize this matrix



  - Sort in descending order

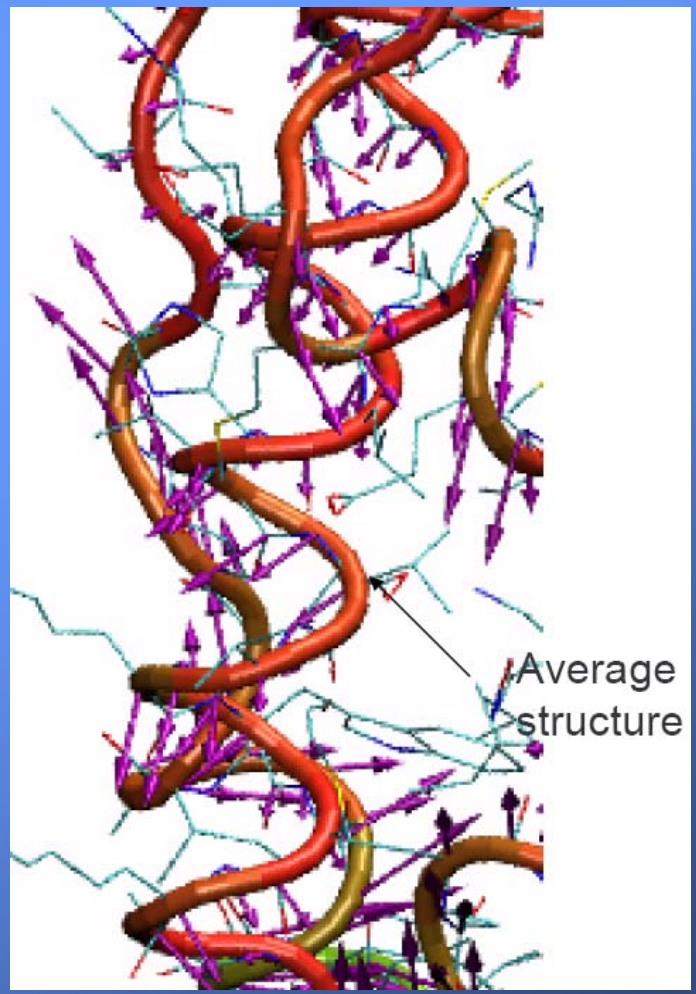
MD cont'd…

projection



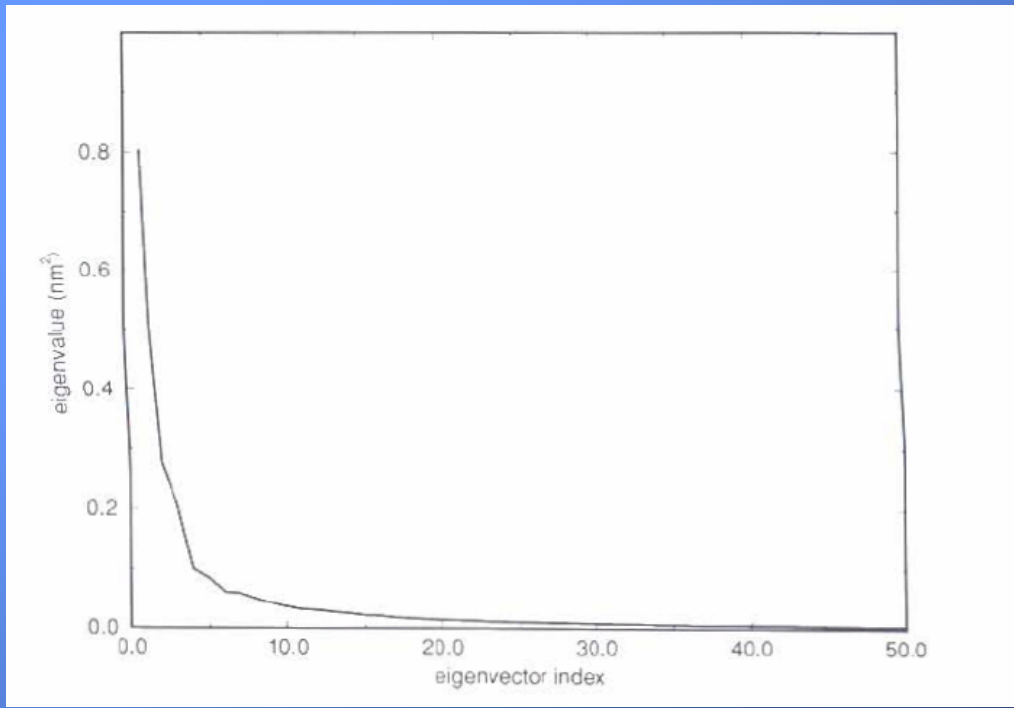
MD cont'd …

- Essential motions determine low energy modes of protein movement
- Atom components of these modes are presented by arrows that show the relative amplitude and direction of the displacement of the atoms from the averaged over MD trajectory structure



MD cont'd …

- Proteins have only few large Eigen values and corresponding eigenvectors - essential subspace of motion
- All others –small high frequency and small amplitude motions can be neglected



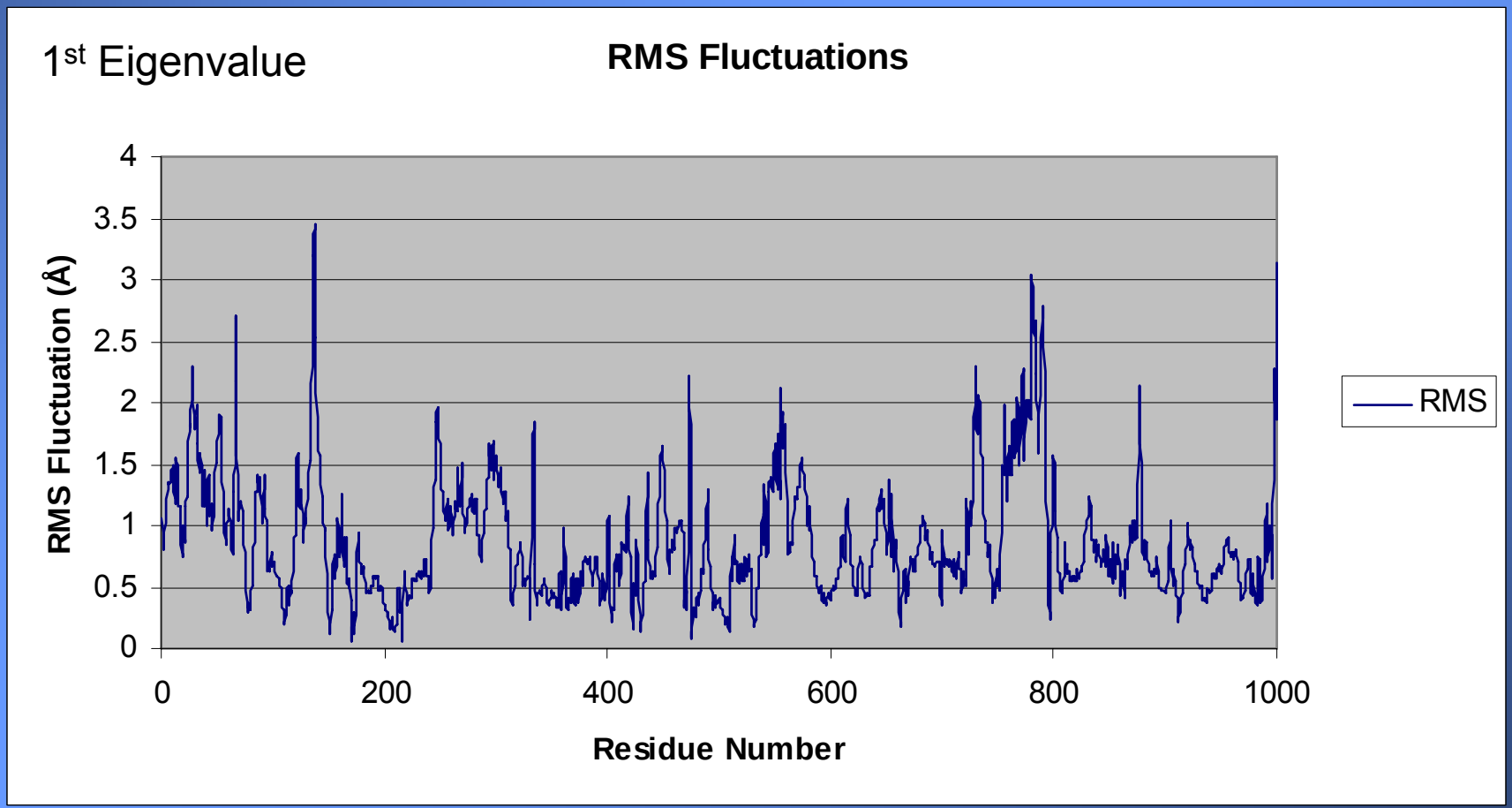
Van alten, 1995

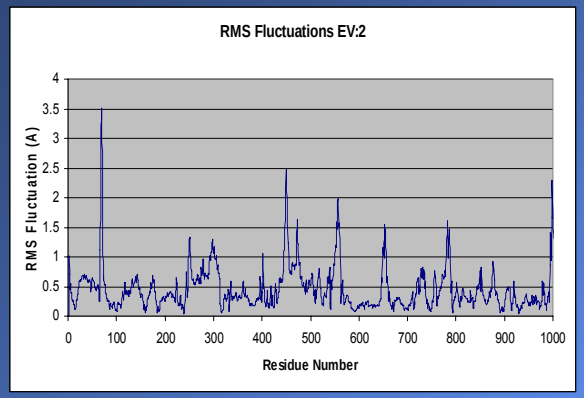
Amber

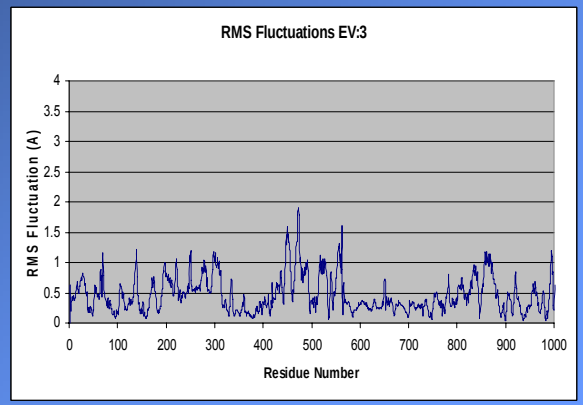
- Has a set of molecular mechanical force fields
- A package of molecular simulation programs
- LEaP
  - X-windows-based program
  - For building amber coordinate and parameter/topology input files
- ptraj
  - Analyze MD trajectories
  - Calculate RMS fluctuations

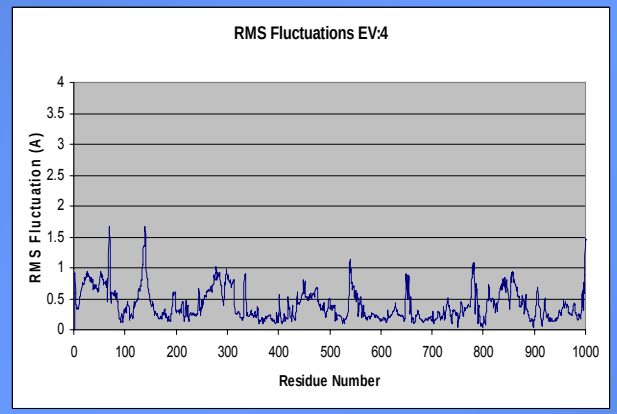
RMS Fluctuations

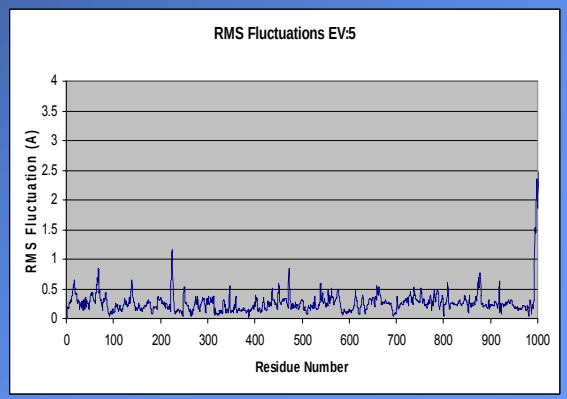
- First 10 Eignevalues

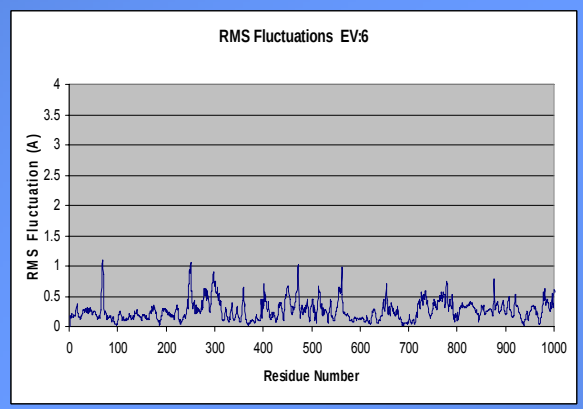


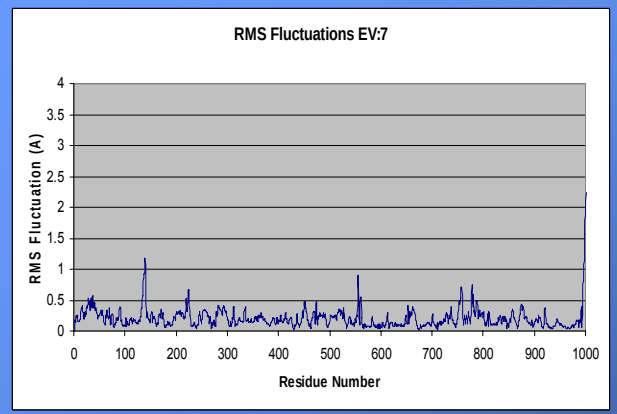

RMS Fluctuations EV:2
RMS Fluctuation (A)
Residue Number

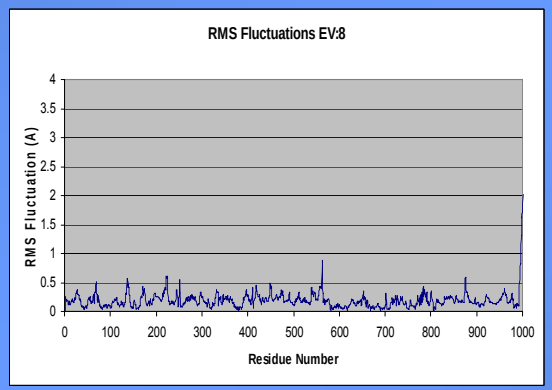

RMS Fluctuations EV:3
RMS Fluctuation (A)
Residue Number

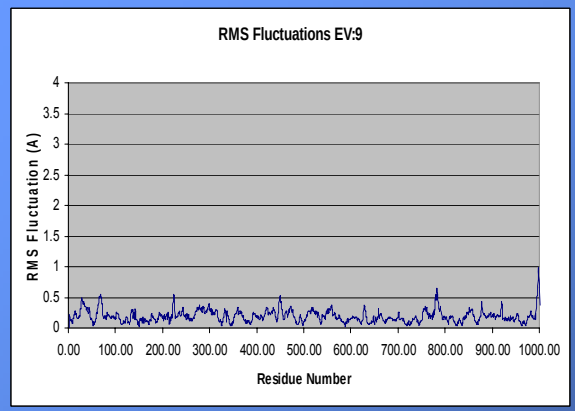

RMS Fluctuations EV:4
RMS Fluctuation (A)
Residue Number

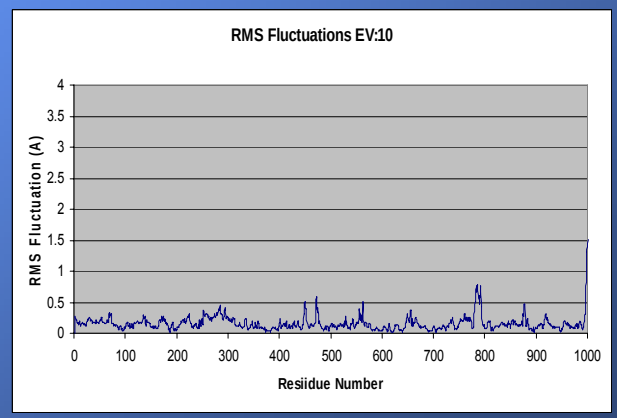

RMS Fluctuations EV:5
RMS Fluctuation (A)
Residue Number


RMS Fluctuations EV:6
RMS Fluctuation (A)
Residue Number

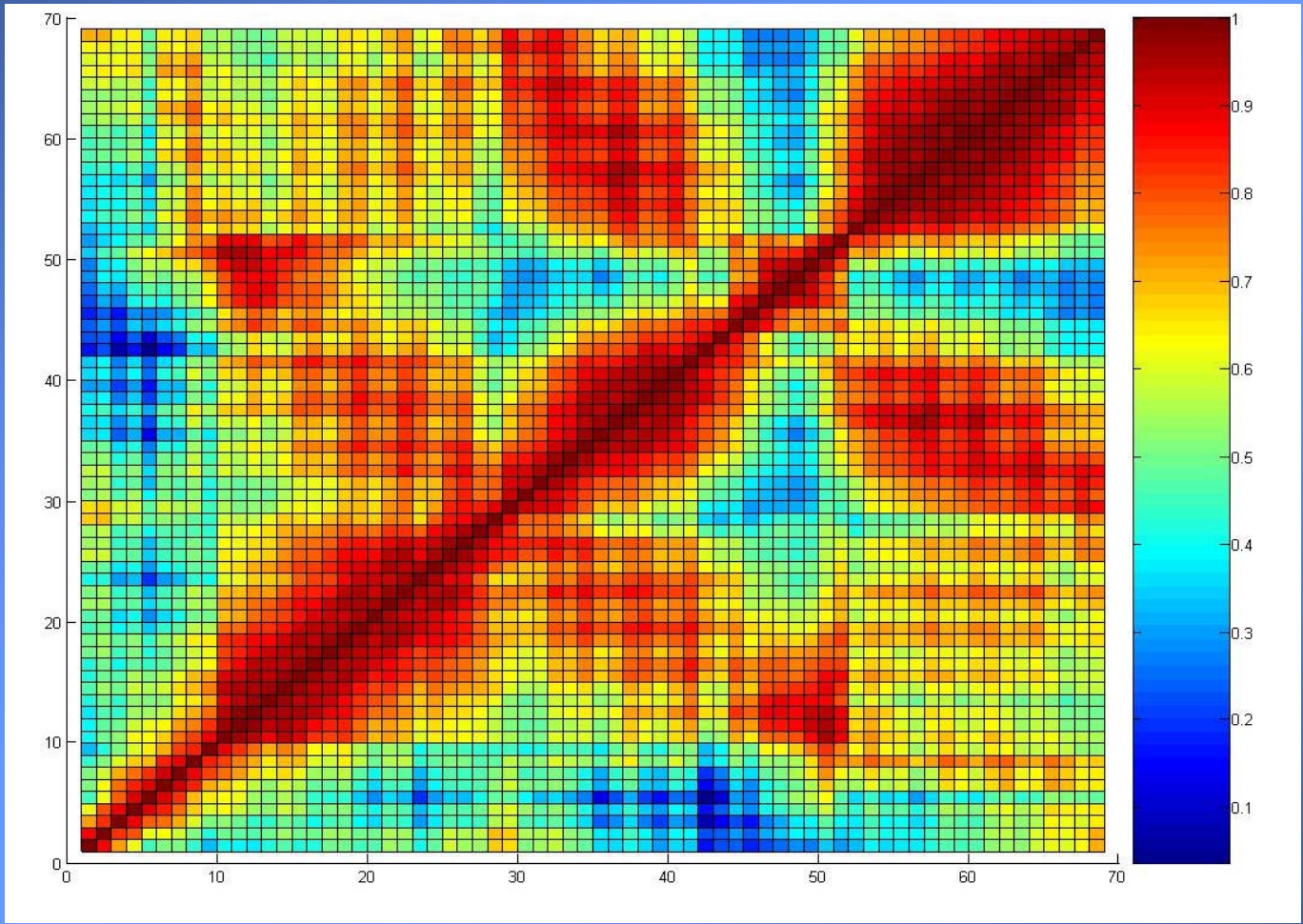

RMS Fluctuations EV:7
RMS Fluctuation (A)
Residue Number


RMS Fluctuations EV:8
RMS Fluctuation (A)
Residue Number


RMS Fluctuations EV:9
RMS Fluctuation (A)
Residue Number


RMS Fluctuations EV:10
RMS Fluctuation (A)
Resiidue Number

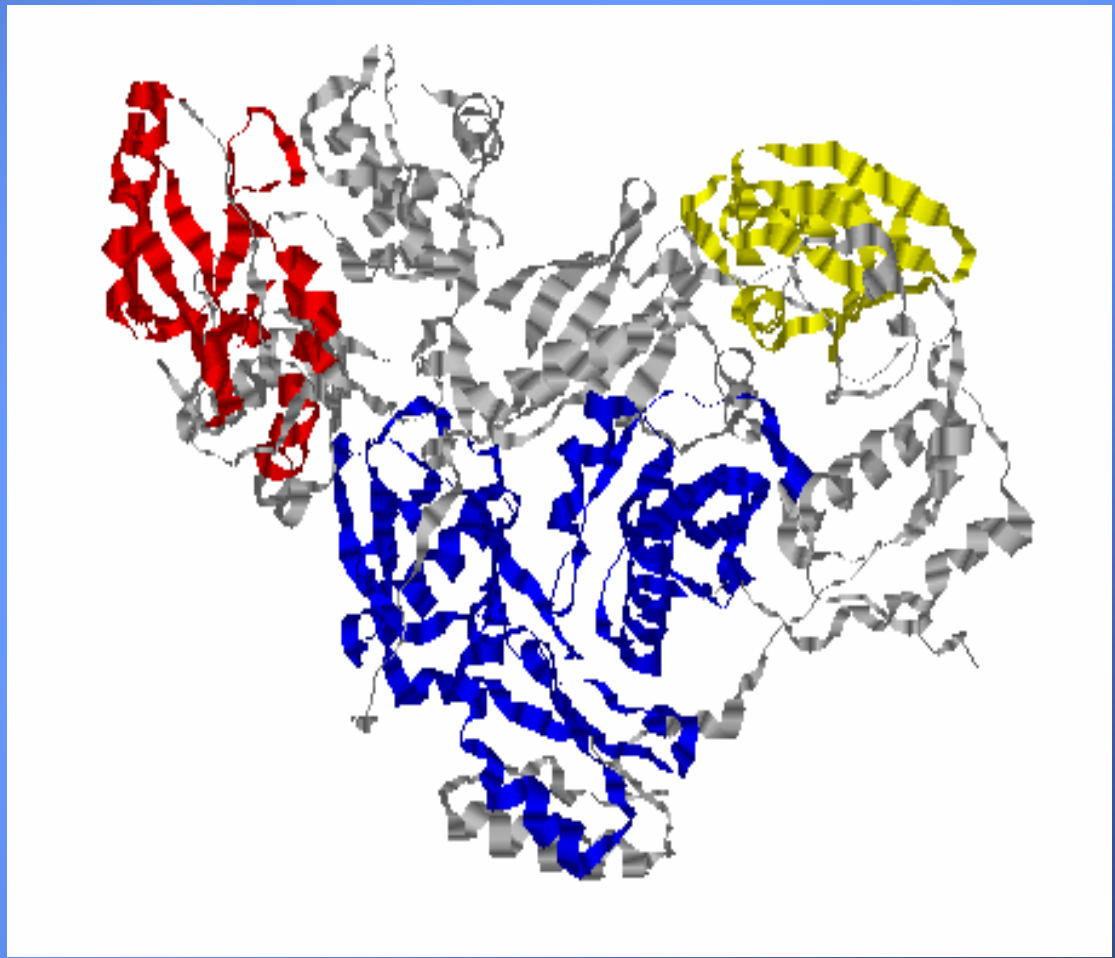
Correlation Matrix



Thumb region: residues 243-311

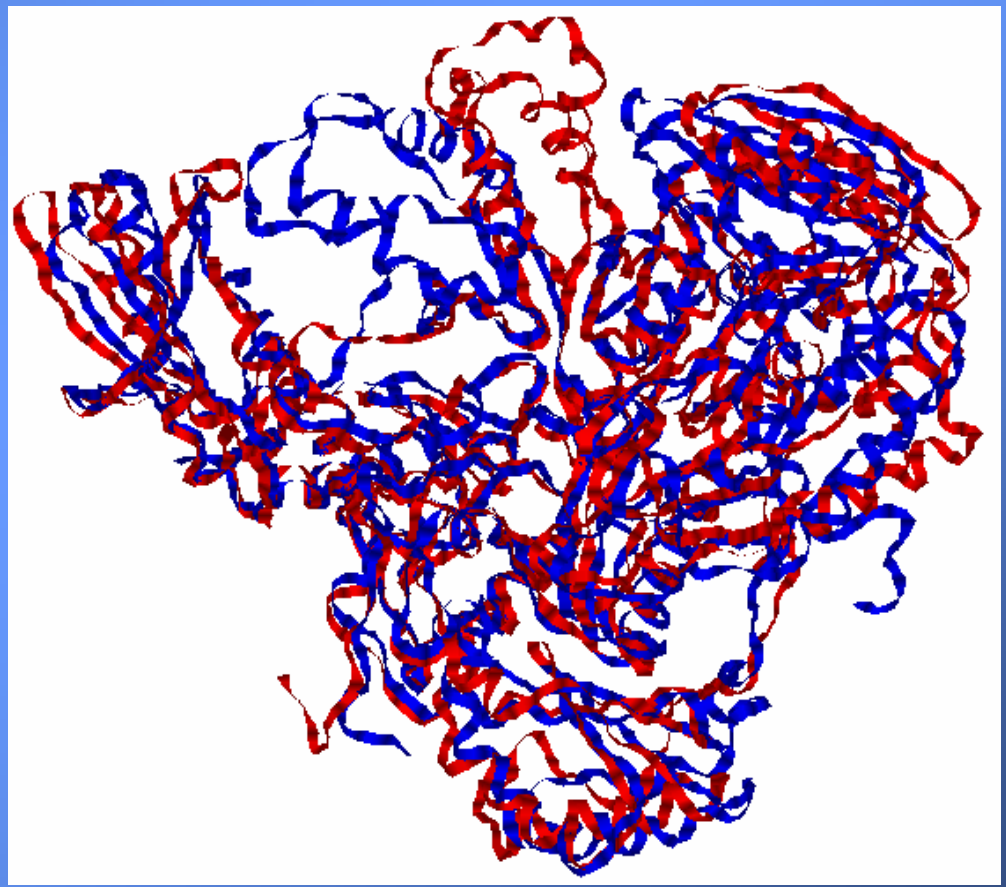
HIV-1 RT from First Analysis

- Rigid cluster 1 (blue)
- Rigid cluster 2 (red)
- Rigid cluster 3 (yellow)



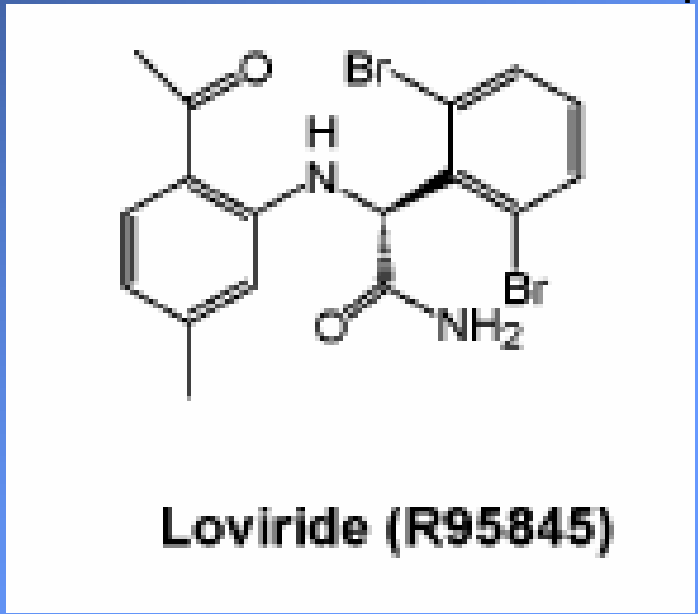
Flexibility of P66 thumb

- Unliganded RT (blue)
  - P66 thumb subdomain folded into DNA binding cleft
- DNA bound RT (red)
  - P66 thumb subdomain in upright position

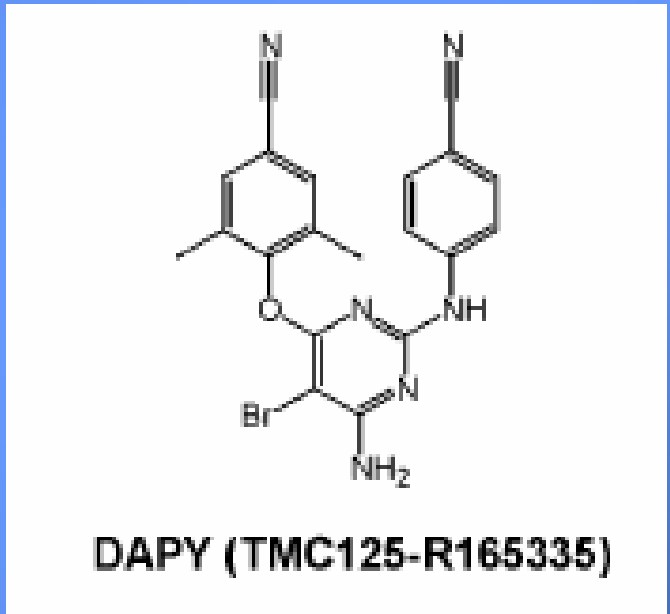


Non-Nucleoside RT inhibitors:

binding of may alter conformation of template primer
& Inhibit polymerization



Loviride (R95845)



DAPY (TMC125-R165335)

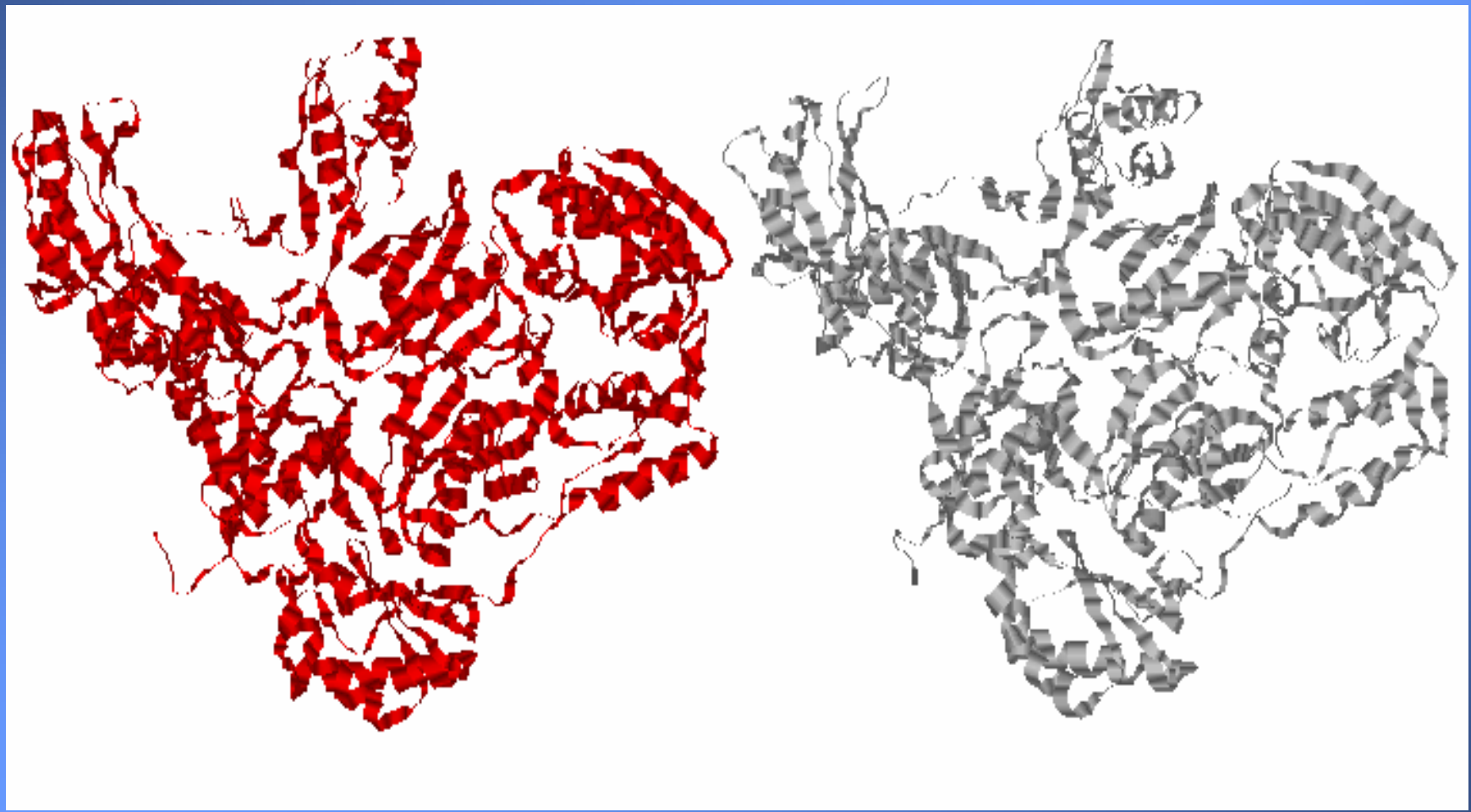
1st Generation

Effective against wild type
HIV-1 but not mutants

2ND Generation

(more flexible)
Binds RT in many
conformations & escapes
drug-resistance mutations

Complex of HIV-1 RT with dsDNA (red)
Complex of HIV-1 RT and with TMC125 (grey)



Summary

- Molecular Dynamics
- Understanding the conformation flexibility of HIV-1 RT is essential in
  - Controlling mechanism of polymerase
  - Binding of inhibitors
  - Developing more efficient drugs
- Lower infection rates slowly in different populations – decrease AIDS fatality

Acknowledgements

- Dr. Maria Kurnikova
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Questions?


köszönöm
תודה!
dėkuji
mahalo
고맙습니다
thank you
merci
谢谢
danke
Ευχαριστώ
شكرا
どうもありがとう
gracias