HIV1 Protease

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Structure

- Homodimer; each monomer = 99 amino acids
- Monomers have identical conformations
- Monomers stabilized by
  - Aliphatic residues
  - Noncovalent interactions
  - Hydrophobic packing of side chains
  - Interactions involving catalytic residues
- Each monomer has 2 cysteine residues (25, 29)
- Secondary structure is one α helix and two antiparallel β sheets
- Each monomer has 1 extended “loop” comprising of residues 46-54

->PyMol

Function

- HIV1 protease is an **aspartyl protease**
- …are characterized by conserved sequence of Asp-Thr-Gly
- HIV protease is an exception because most aspartyl proteases are monomeric enzymes consisting of two-domains
- HIV1 cleaves specific dipeptide bonds at the target or substrate
    - All retroviruses consist of at least three genes that are required for viral replication: Gag-Pol-Env
    - HIV1 protease is required for cleavage of these viral precursors

Active Site

- Active site formed at dimer interface and is created in a cleft between the two domains as part of a 4-stranded β turn
- Each monomer contributes one Asp-Thr-Gly triad (aa 25, 26 and 27)
- Asp 25 from each monomer holds a water molecule by forming hydrogen bonds
- Two aspartates are said to form a “catalytic diad”; one of these exhibits an unusually low pKa of 3.3 and the other a high pKa of 5.3
- This triad interacts with the amide bond to be cleaved in polypeptide substrate

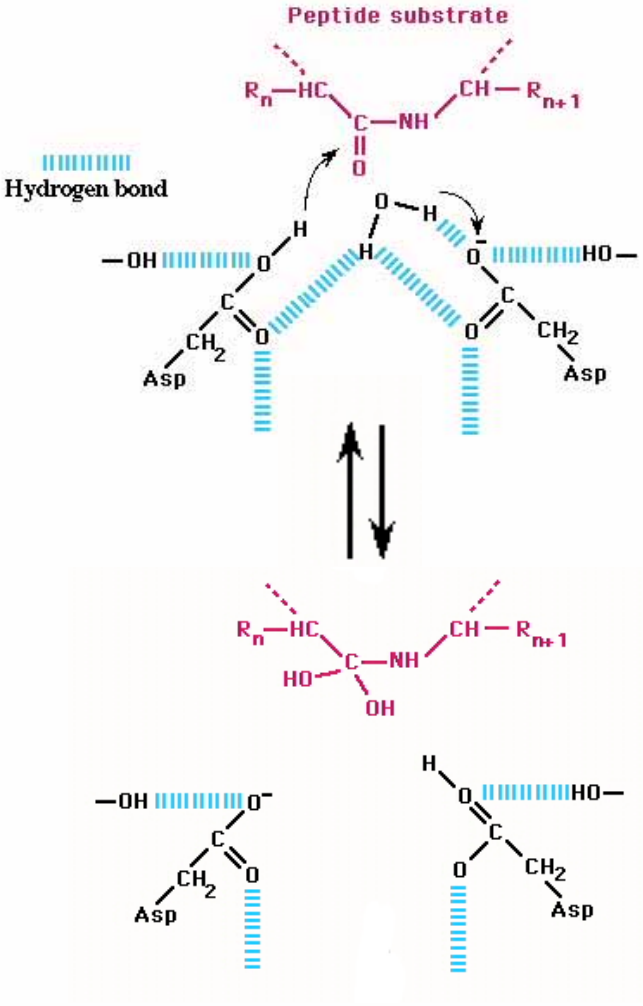
->*PyMol*

Catalytic Mechanism of Aspartic Proteinases

Different pK's of the aspartates leads to one acting as a general acid catalyst to protonate the carbonyl oxygen, and the other acting as a general base…

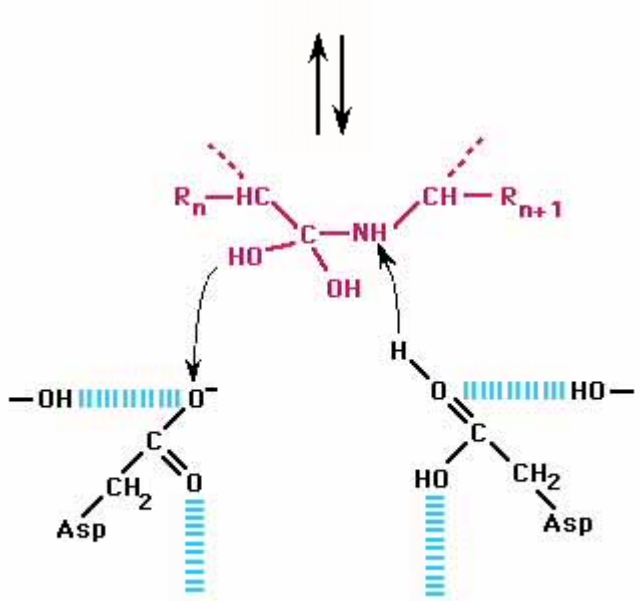
to pull the proton from water.

nucleophilic attack by the water's oxygen to the carbonyl's carbon forms an amide dihydrate intermediate



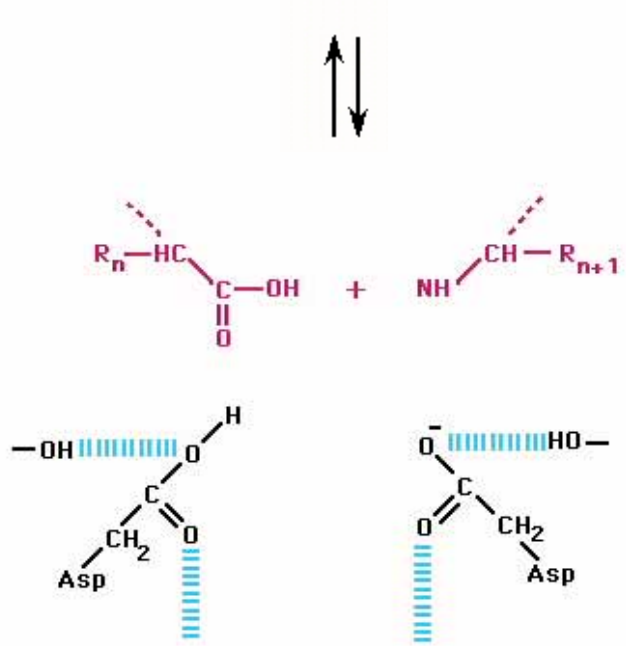
Catalytic Mechanism of Aspartic Proteinases

Amide dihydrate breaks down ...



Catalytic Mechanism of Aspartic Proteinases

…to form the cleaved products.



The “flaps”

•The mobile flap, residues 46-54, contains three characteristic regions: side chains that extend outward (Met46, Phe53), hydrophobic chains extending inward (Ile47, Ile54), and a glycine rich region.
•A water molecule binds to Ile50 from the interior of the cleft when the protein is unliganded
•The flaps are closed when the active site is occupied by a ligand.
•Crystal structures reveal that even the semi-open flaps block access to the active site, indicating that the flaps are mobile in solution.

->*PyMol*

For computer scientists?

- The flap movements are central to the function of the enzyme, yet determining how these flaps move at an atomic level has not been experimentally possible.
- Flaps of HIV-1 protease can be calculated to completely open during a 10 ns solvated molecular dynamics simulation.
- “Opening” movement is on the time scale observed by NMR relaxation data.
- The highly flexible tips of the flaps curl back into the protein and bury many hydrophobic residues.

The "flaps" – predicting movement

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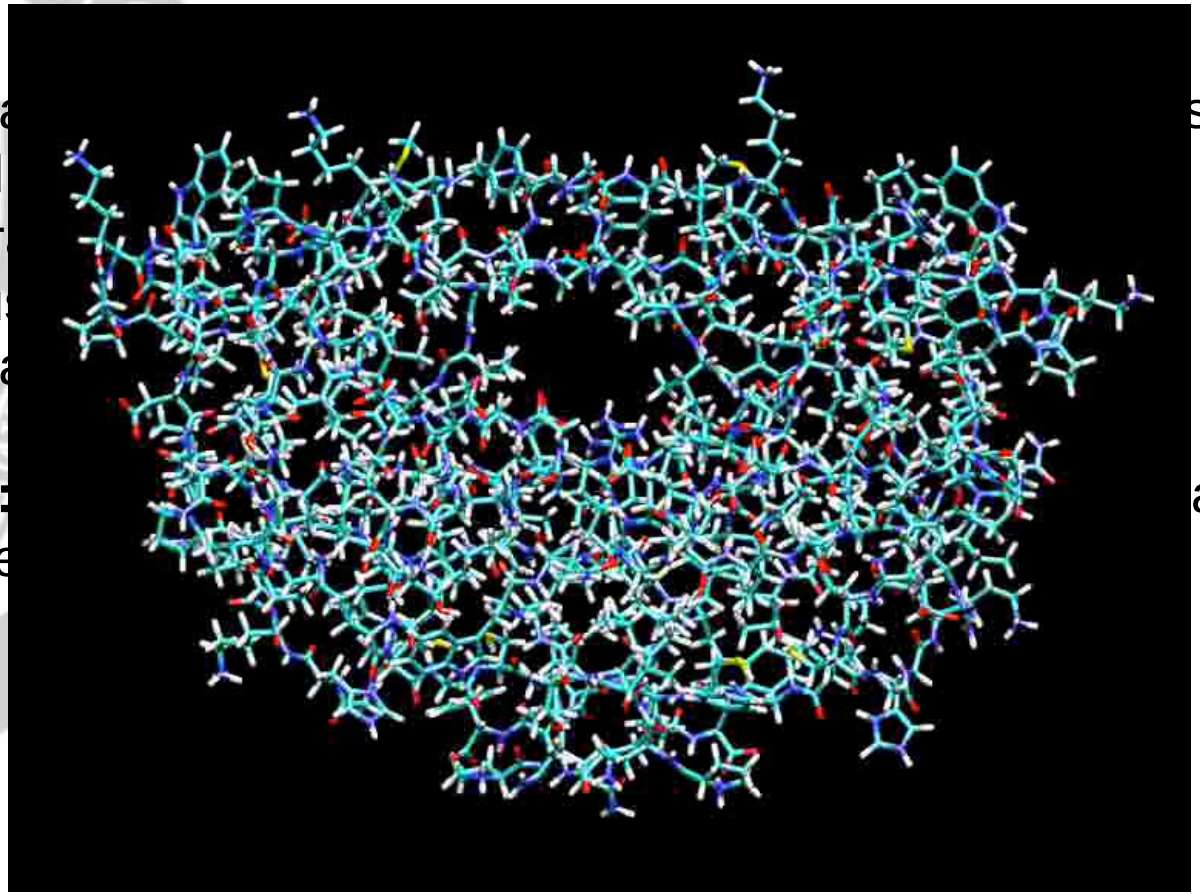

Kavraki, et al., Rice University

The "flaps" – predicting movement

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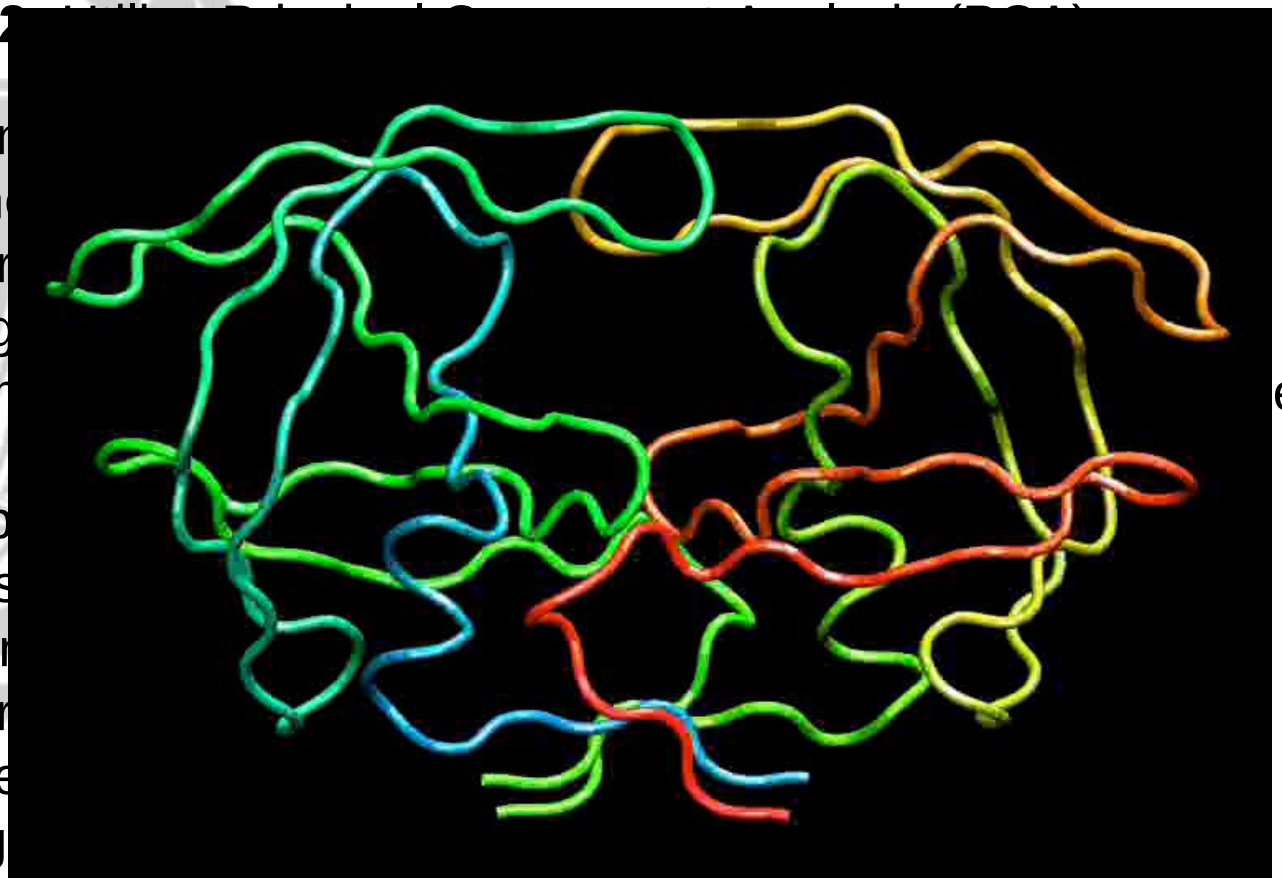

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Kavraki, et al., Rice University

Conclusions

HIV1 Protease :

Aspartyl protease
Monomer Aspartates cleave viral precursors
Flexible flaps “expose” active site

Prediction of flap flexibility: use computer modeling

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