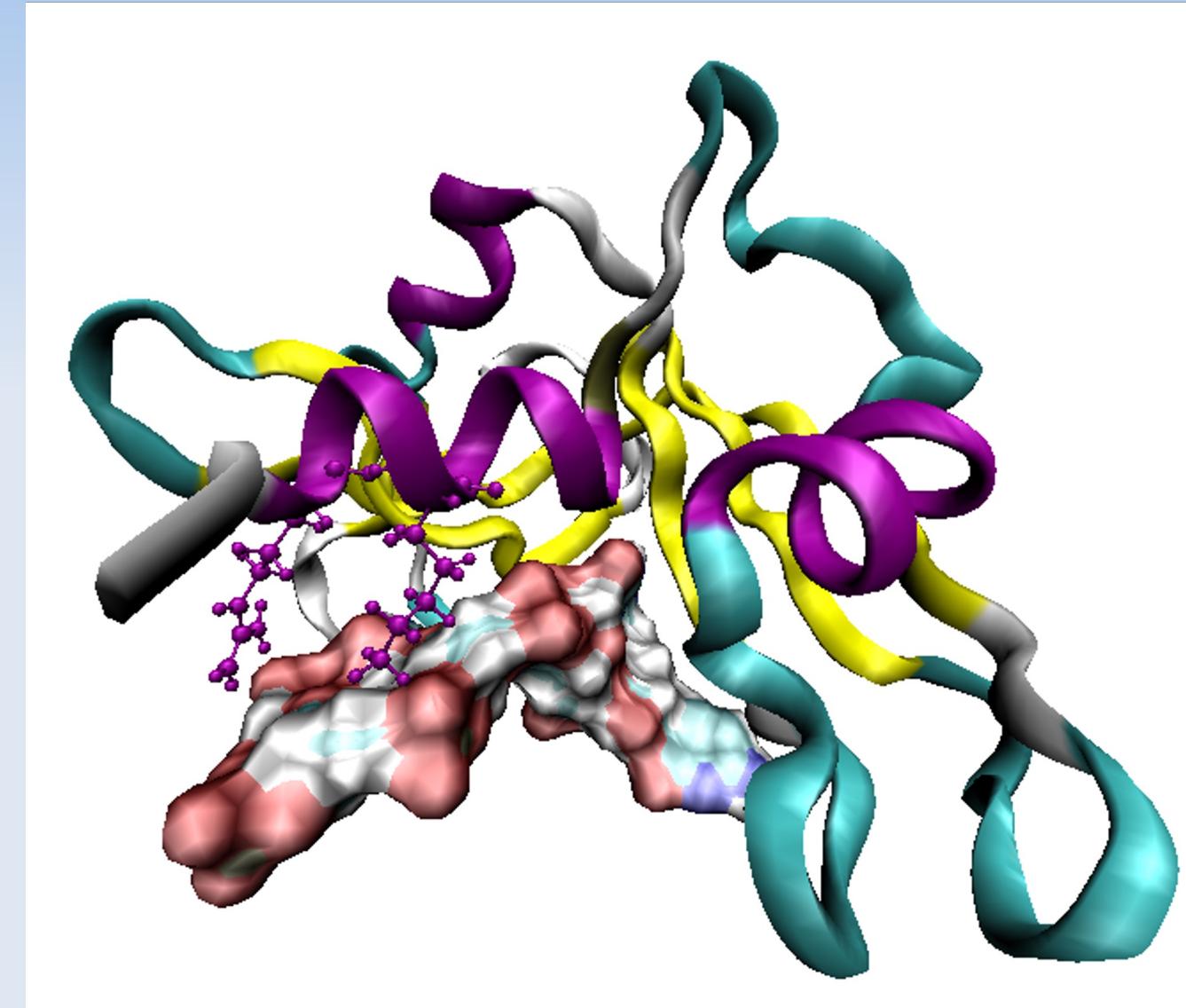


Human Pancreatic Ribonuclease

Molecular Simulations to compare it with Ribonuclease A

*Group Meeting
Xavier Prat-Resina*

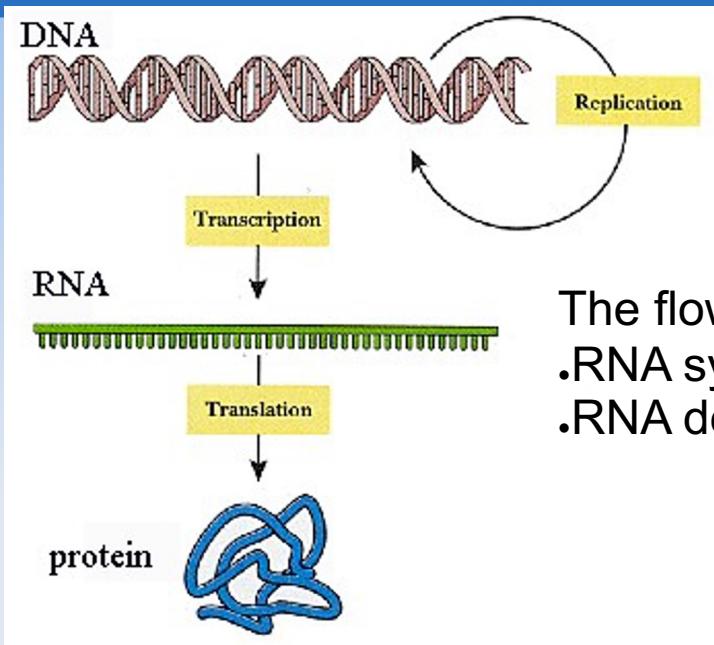
April 20th 2007



Outline

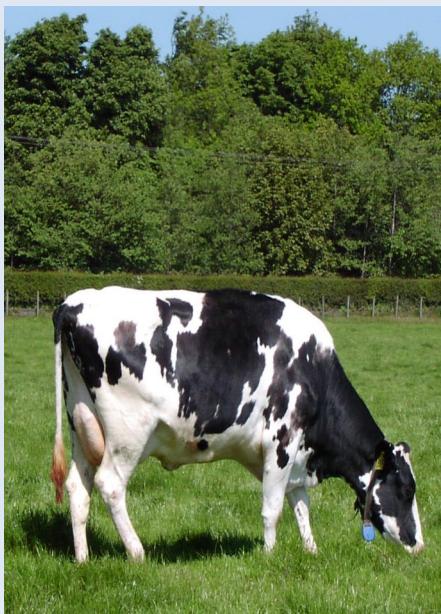
- Introduction to Ribonuclease Structure and Reactivity
- Experimental Results: HP-RNase vs RNase A
- Setup for Molecular Simulations
- Structure Analysis of Molecular Dynamics
- Free Energy Calculations
- Conclusions and Future work

1. Introduction: Struct. & React.

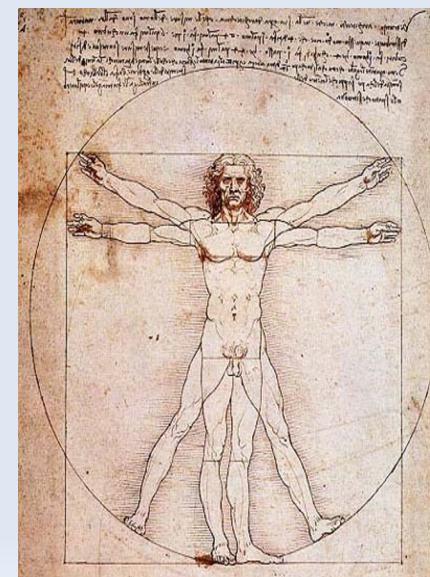


The flow of RNA is controlled by two classes of enzymes

- .RNA synthesis: RNA polymerases
- .RNA degradation: RNA depolymerase or often called "ribonucleases"



Bovine Pancreatic Ribonuclease A (Rnase A)
.Crystallized 50 years ago
3rd enzyme with X-ray struc
(over 70 sets entered in PDB)
.The 1st protein obtained by total synthesis
.Landmark work on folding, stability and chemistry. (2 Nobel prizes)

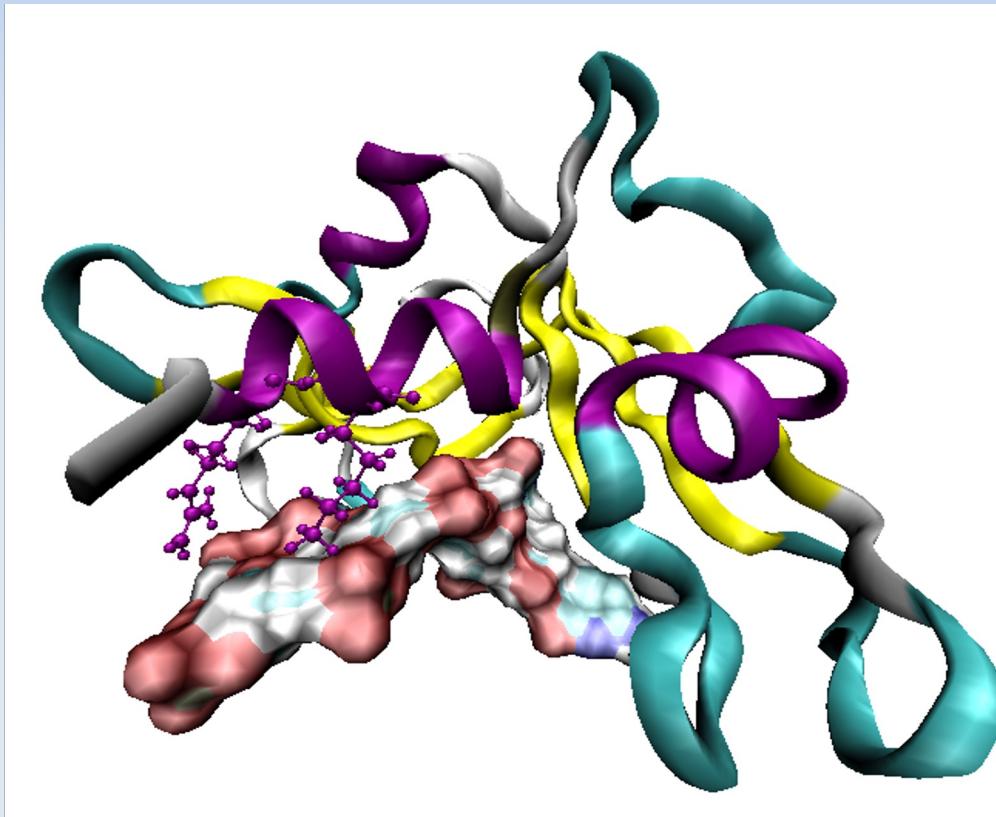


Human Pancreatic Ribonuclease (HP-Rnase)
128 residues
Rnase A homologue

1. Introduction: Struct. & React.

Structure:

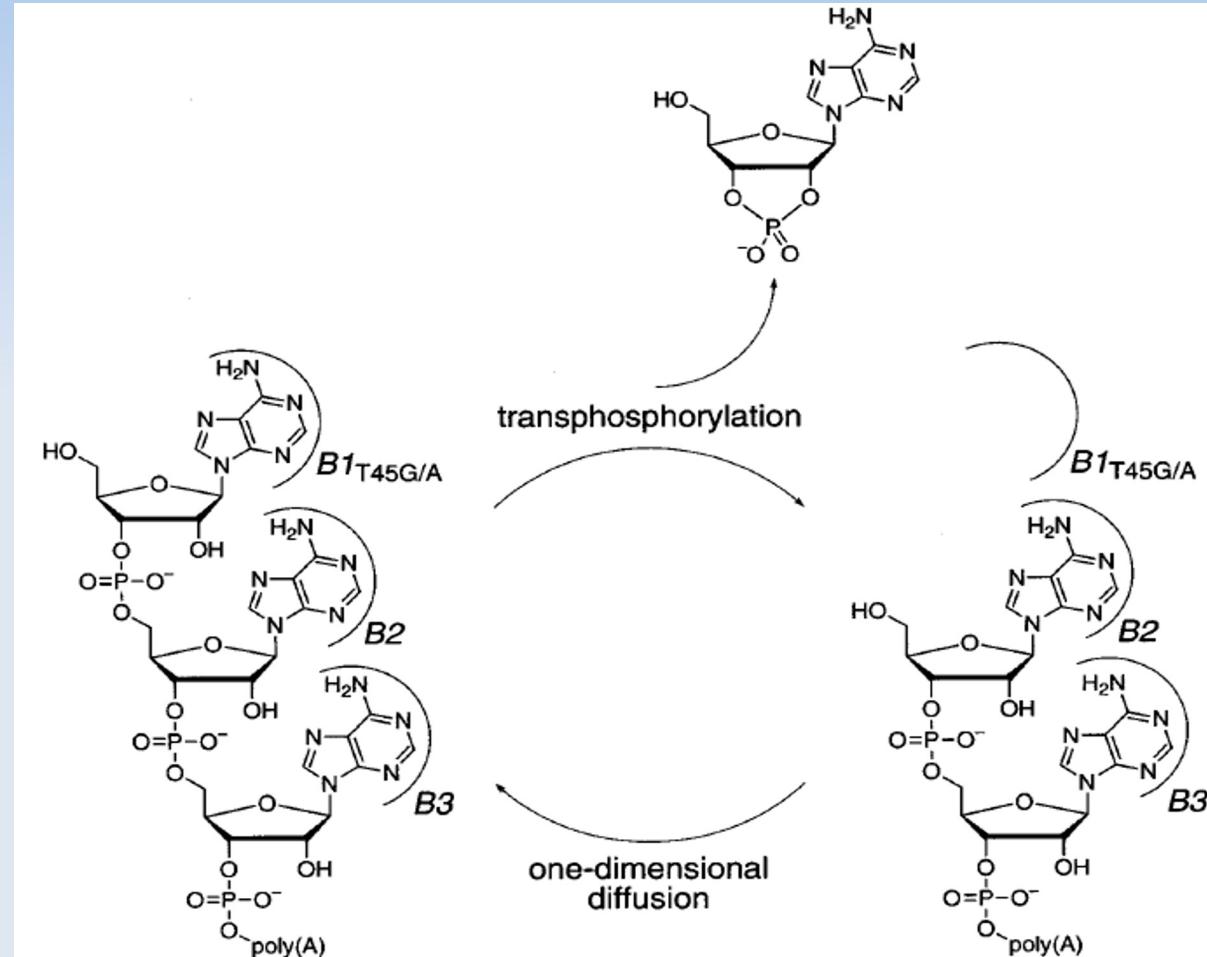
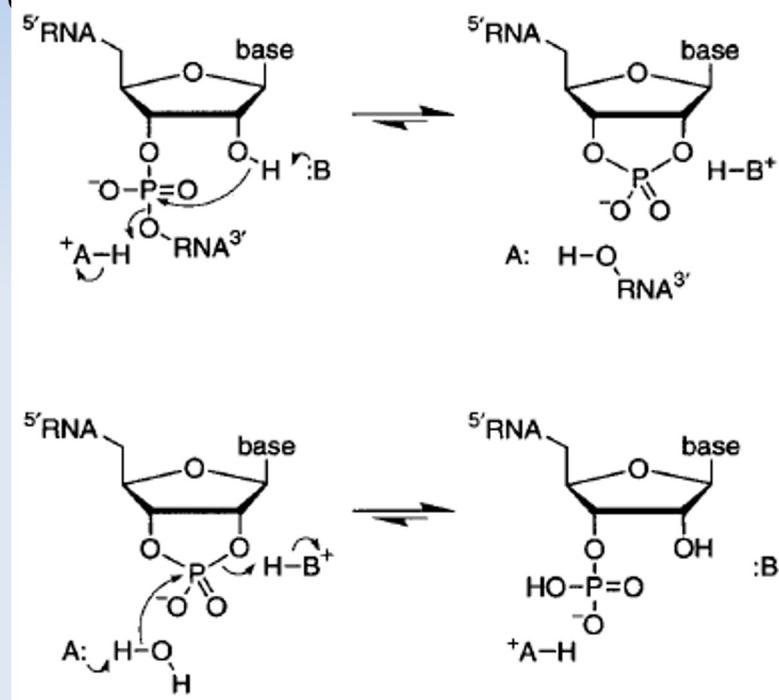
HP-RNase with
tetra-nucleotide
(4 cytidine)
in endo
coordination



The difference between HP-Rnase and Rnase A is that the former has 2 more positively charged residues at the N-terminal: Lys6 and Arg4

1. Introduction: Struct. & React.

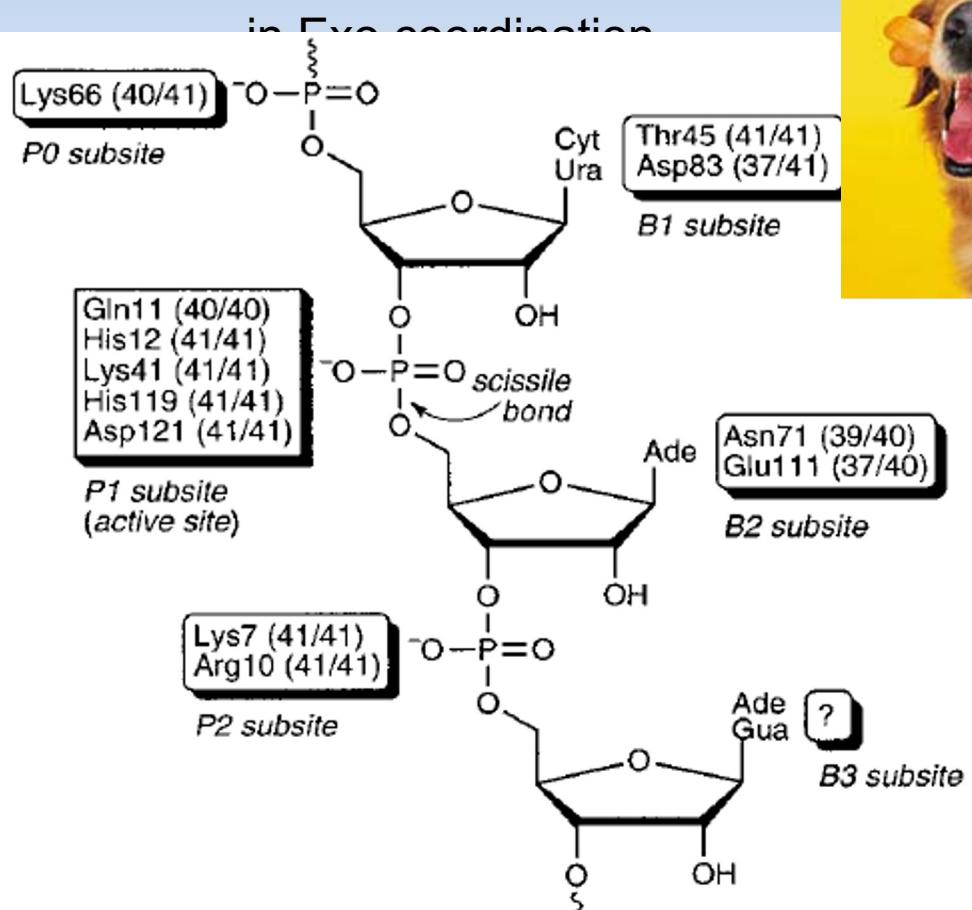
Enzymatic reaction



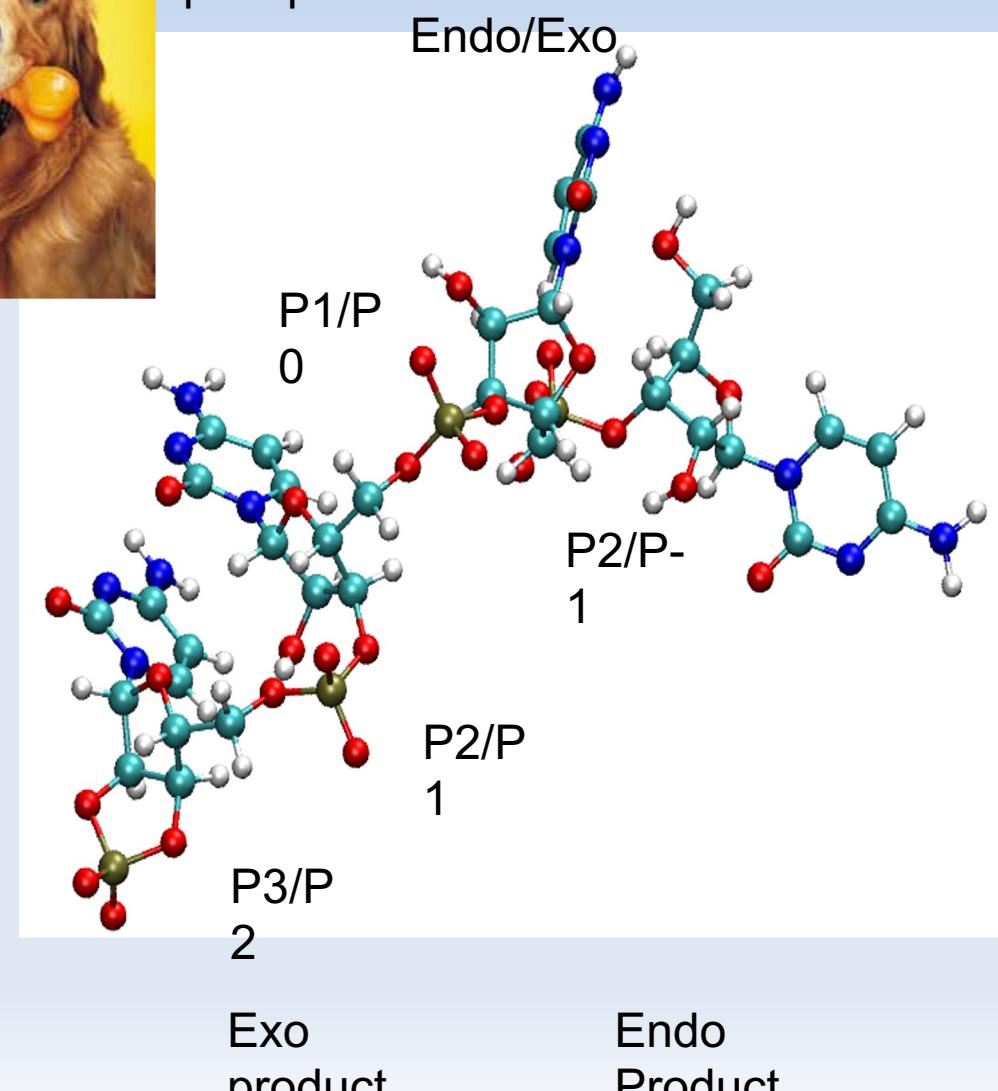
The substrate for our simulations: tetra-cytidilic acid with a cyclic phosphate

1. Introduction: Struct. & React.

Active site and binding sites



4-Cytidine with cyclic phosphate



Exo product

Endo Product

Outline

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2. Experiment: HP-RNase vs RNase A

Experimental results:

- .Human Pancreatic Ribonuclease (HP-Rnase) cleaves polymeric substrate with a marked endonuclease preference compared to Ribonuclease A
- .However both enzymes catalyze endo and exo coordinated substrates.
- .To emulate RNase A a mutant is studied: R4A, K6A, Q9E, D16G, S17N.
- .The ratio between exo and endonucleolytic product is 1.35 in HP-RNase and 2.15 for the mutant.

Experimental Hypothesis

The difference between Human (HP-RNase) and bovine (RNase A) is the Arg4 and Lys6 which reinforce the P2 binding or constitute a new P3 binding site. This is the main question

Challenge for us:

- .Figure out the origin of those differences and the role of the positively charged residues present in HP-Rnase in the N-terminal
- .There is no crystal structure for HP-RNase:
 - Structure obtained by Homology (Alex Gomez-Garrido)
- .Molecular Dynamics Simulations and Free Energy Computations
- .We will assume that the differences in reactivity can be explained by the difference in the binding

Outline

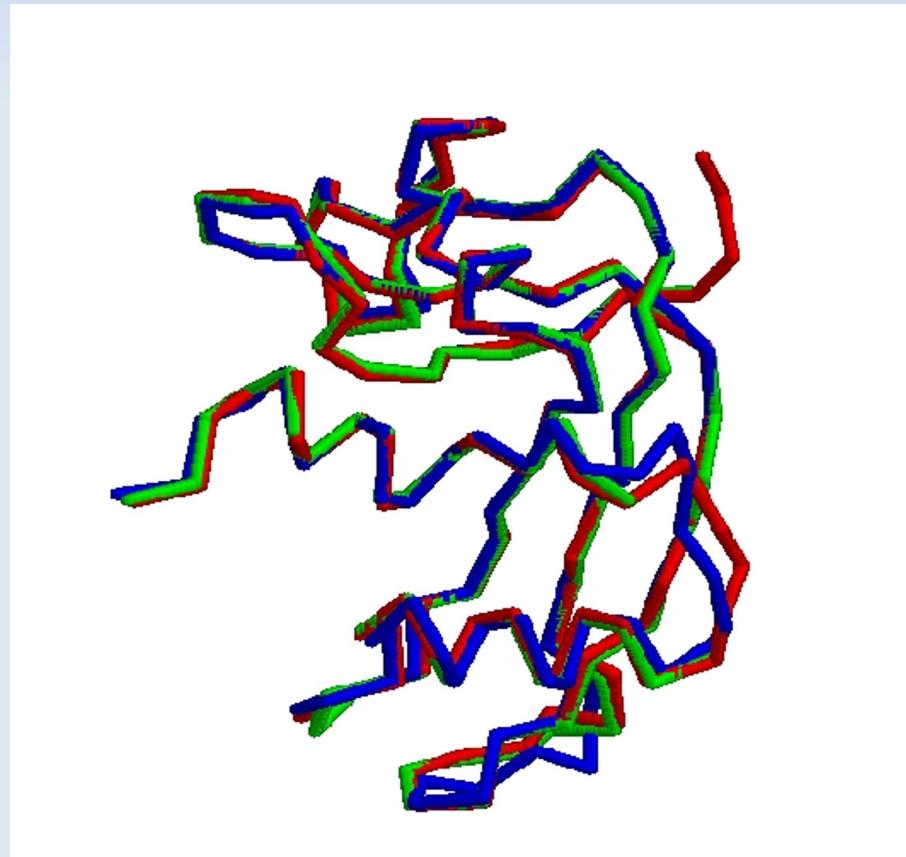
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3. Setup for Simulations

Homology modelling (Alex Gomez-Garrido):

Initial structures: RNase A bovine 1RCN and HP-RNase mutant 1DZA with 4 DNA nucleosides (ATAA)

The structure are all very similar



3. Setup for Simulations

Setup for HP-RNase

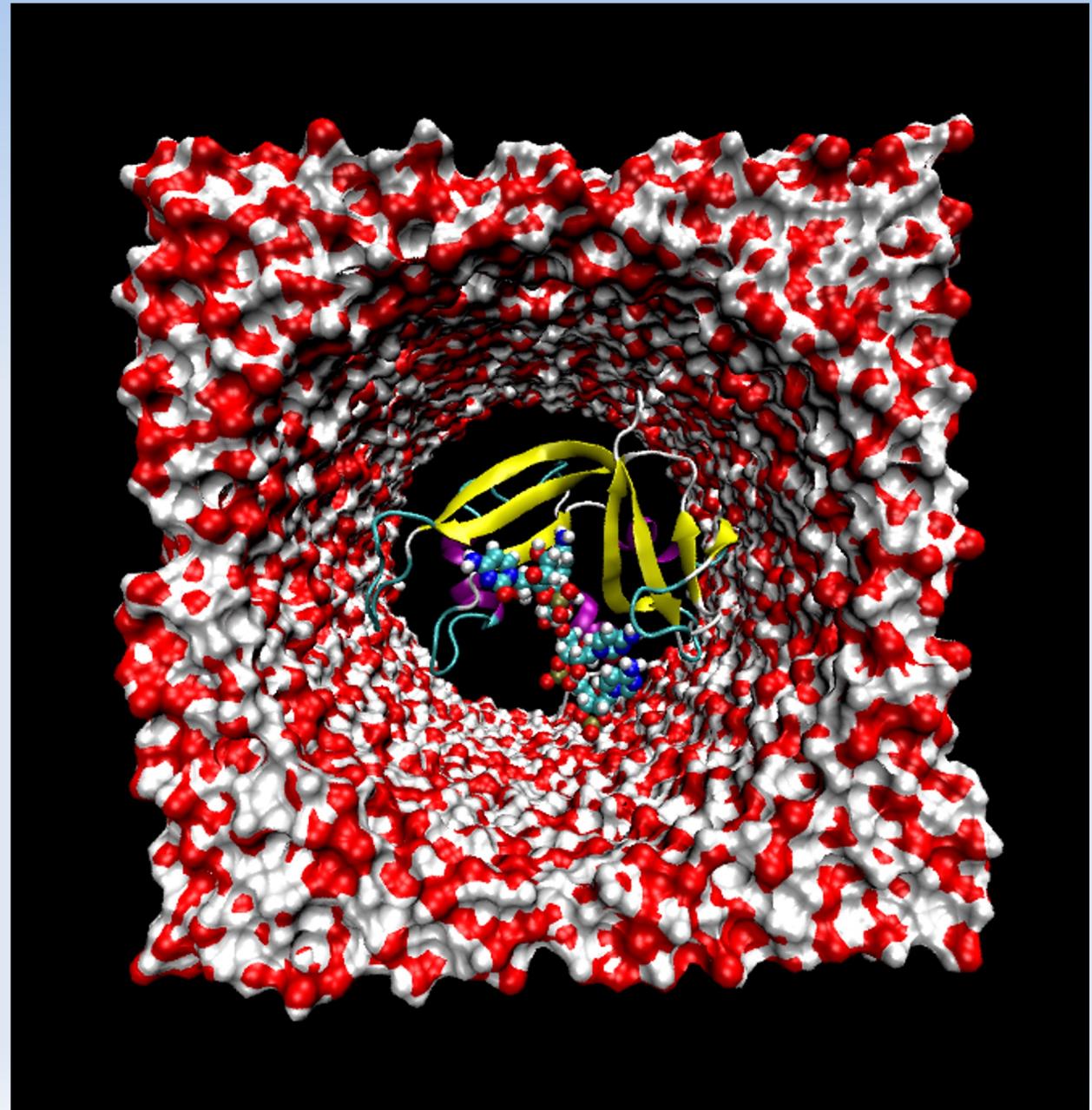
- .A cube of water of 65 Angstr (~30,000 atoms)
- .Charmm forcefield, no extra parameters. NAMD/Vmd soft.
- .PBC with Particle Mesh Ewald for electrostatics
- .2fs timestep, shake, NPT ensemble
- .Progressive heating and unrestriction for almost 1ns
- .Simulation ~5ns
- .4 setups:

Wild Type/ Endo

Wild

Type/Exo

Mutant/Endo



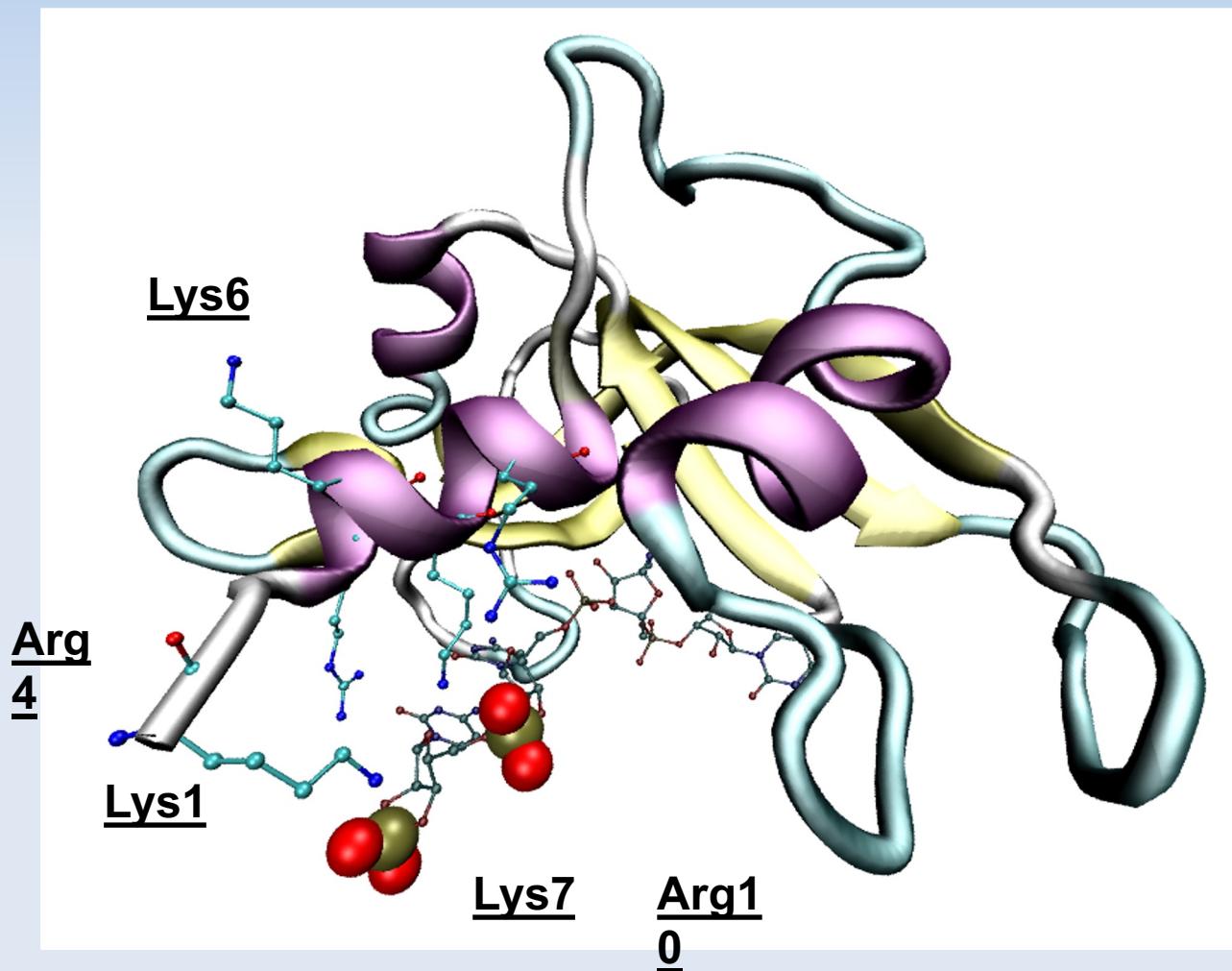
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4. Struct. Analysis of MD

Analysis of important distances

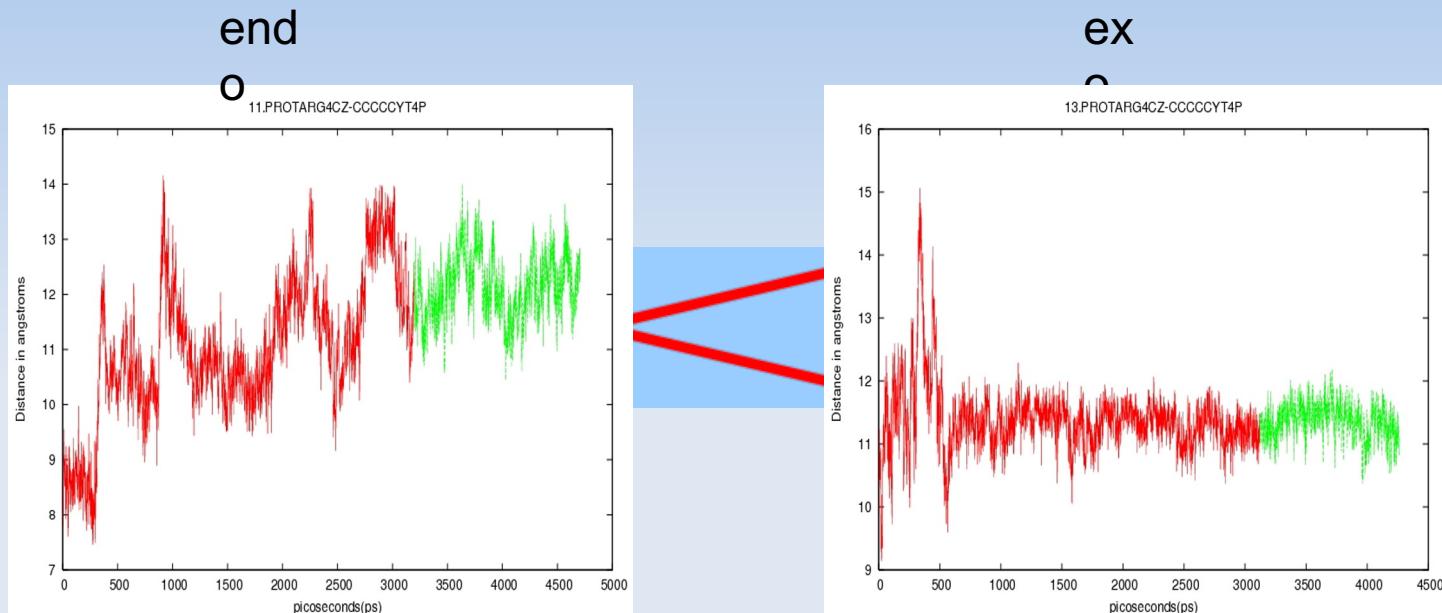
We want to see the role of different residues
Differences between exo/endo and wild type and mutant



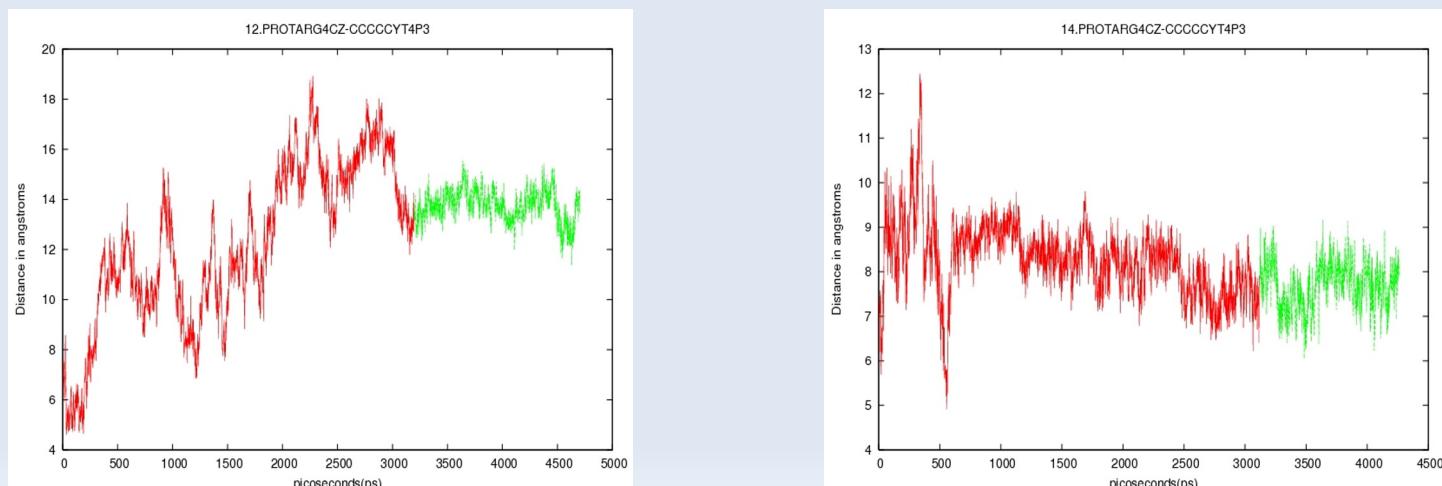
4. Struct. Analysis of MD

Analysis of important distances

Arg4-
P2



Arg4-
P3

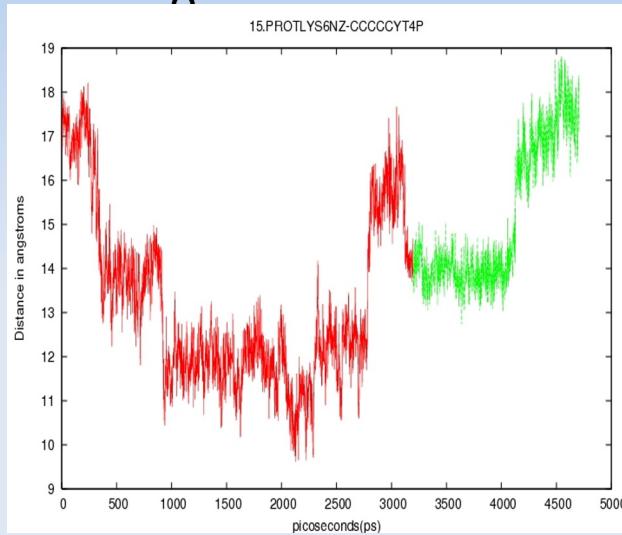


4. Struct. Analysis of MD

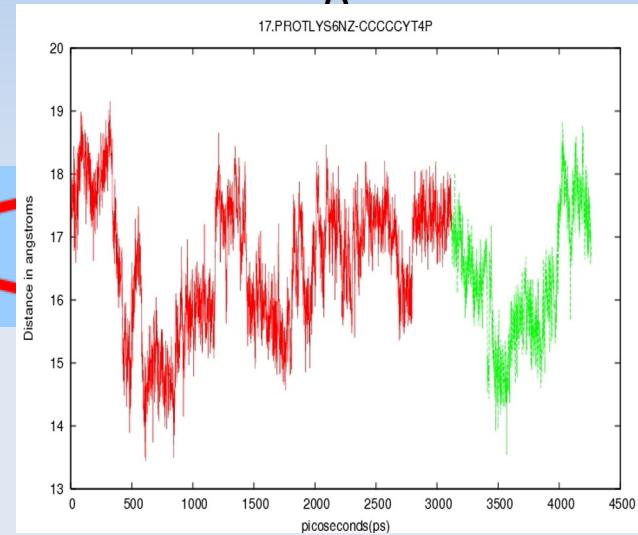
Analysis of important distances

Lys6-P2

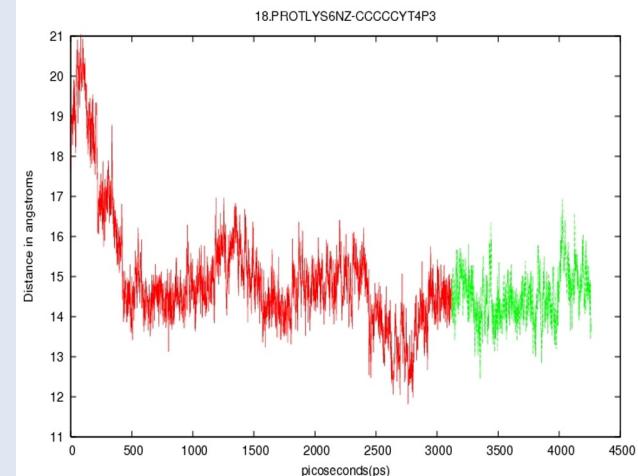
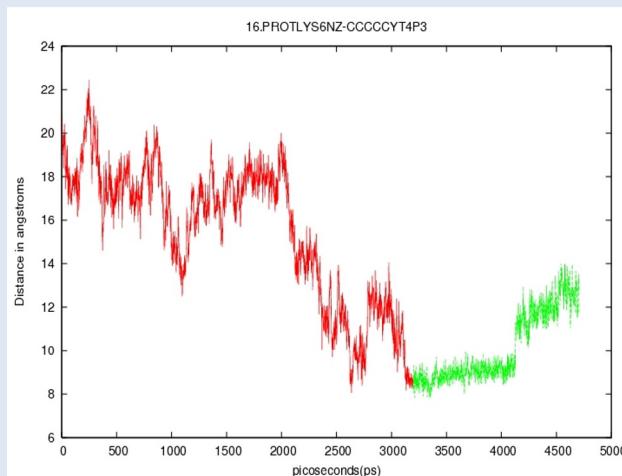
end
C



ex
C



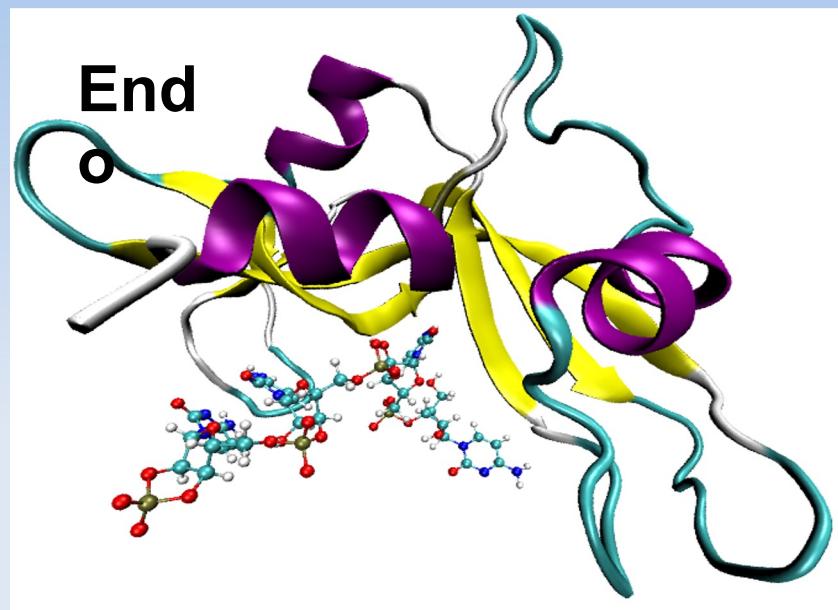
Lys6-P3



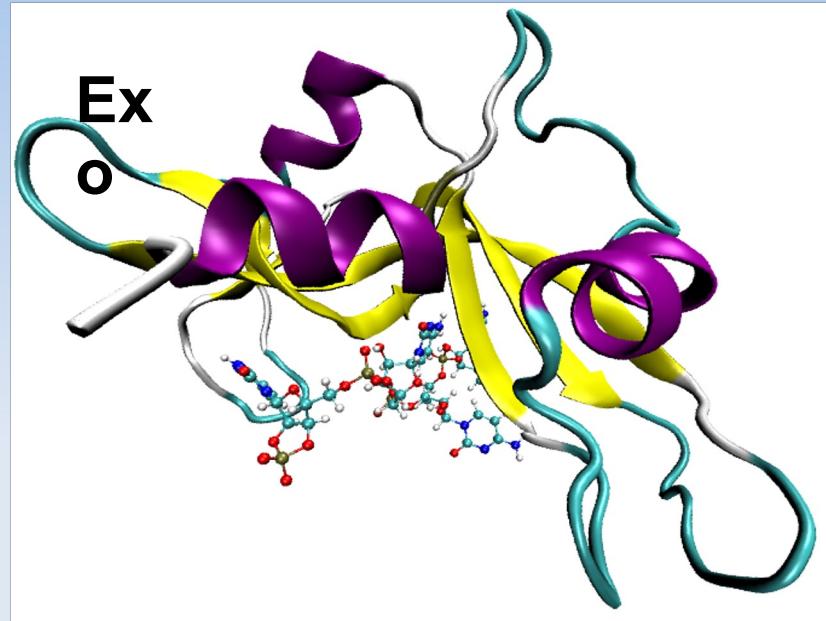
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5. Free Energy Calculations



$\Delta G_{tot}?$



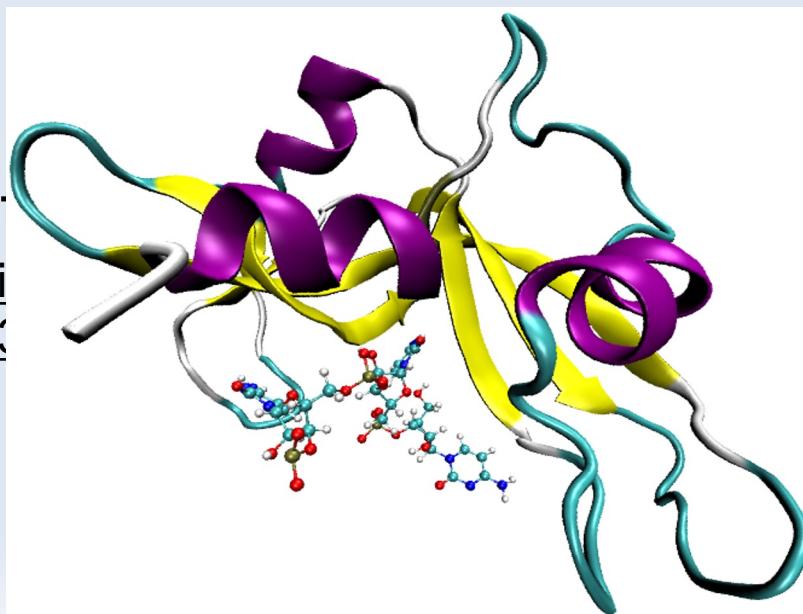
ΔG_1

ΔG_{tot}

The ratio

1.0

*Alchemica
I
pathway*



ant) ?
product is
nt.

ΔG_2

$$\Delta G_{tot} = \Delta G_1 + \Delta G_2$$

5. Free Energy Calculations

Alchemical Free Energy

We run Molecular Dynamics and collect the energy difference between reactant "a" and product "b"

$$\Delta A_{a \rightarrow b} = -k_B T \ln \left\langle \exp \left[-\frac{\mathcal{H}_b(\mathbf{r}, \mathbf{p}) - \mathcal{H}_a(\mathbf{r}, \mathbf{p})}{k_B T} \right] \right\rangle_a$$



To converge the calculation we alchemically transform "a" into "b" scaling the two structures. We define a system as a combination of reactants "a" and products "b"

$$\mathcal{H}(\lambda) = \mathcal{H}_0 + \lambda \mathcal{H}_a + (1 - \lambda) \mathcal{H}_b$$

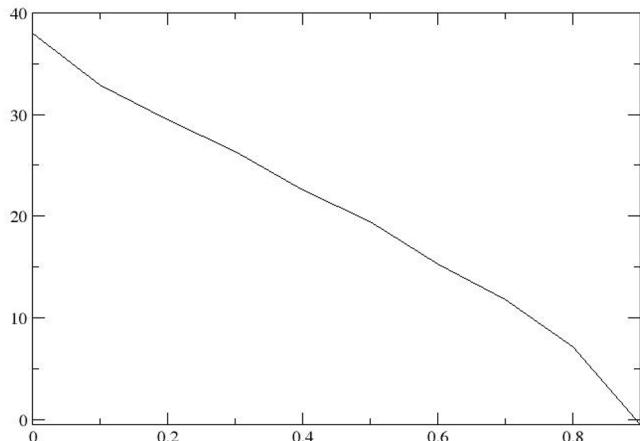
We run several "windows" at different values of λ and combine them (100 ps/window)

$$\Delta A_{a \rightarrow b} = -k_B T \sum_{k=1}^N \ln \left\langle \exp \left[-\frac{\mathcal{H}(\mathbf{r}, \mathbf{p}; \lambda_{k+1}) - \mathcal{H}(\mathbf{r}, \mathbf{p}; \lambda_k)}{k_B T} \right] \right\rangle_k$$

5. Free Energy Calculations

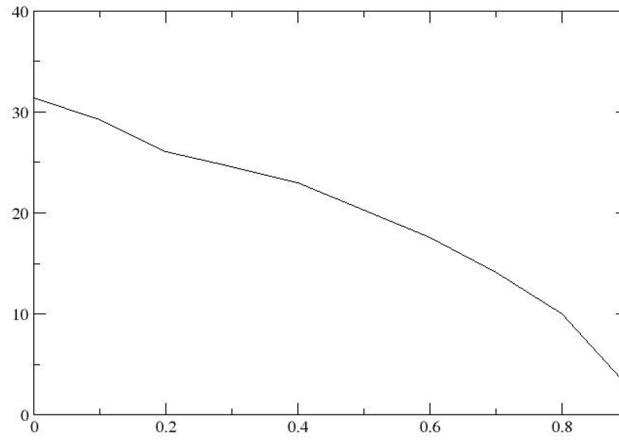
ΔG

1

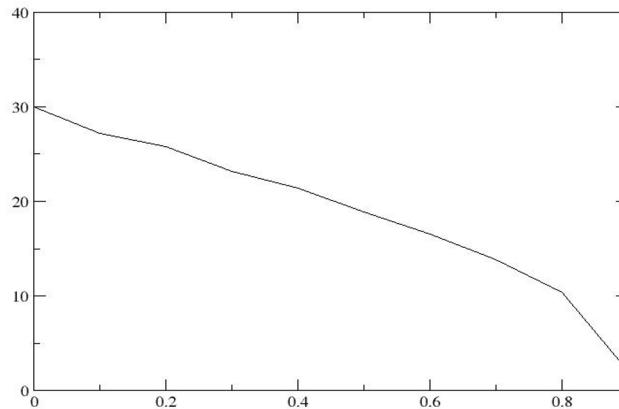
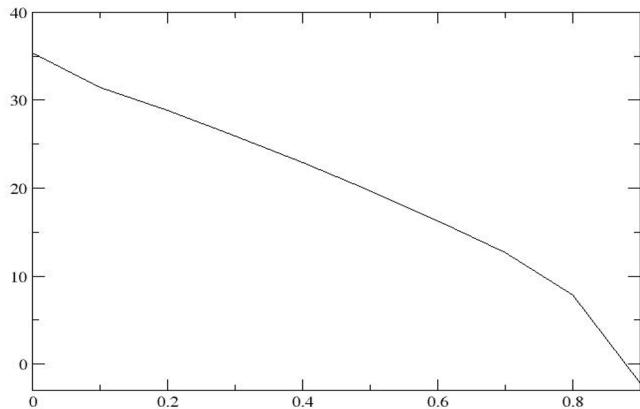


ΔG

2



Wild Type



Mutant

Free Energy
(Kcal/mol)

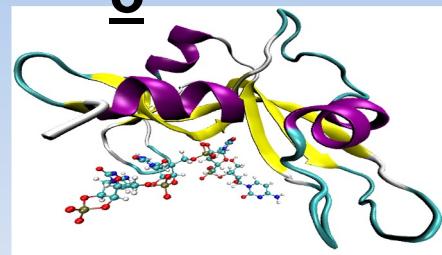
Lambda (0-1)

5. Free Energy Calculations

Wild Type

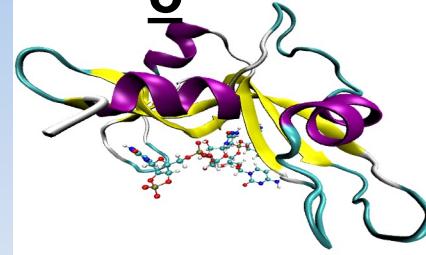
End

o



Ex

o

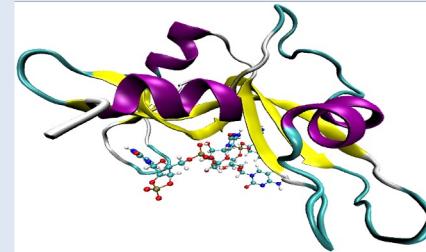
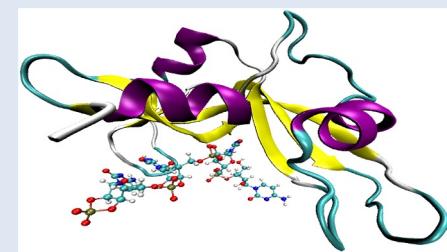


$$\Delta G_{tot} = 202.54 - 199.17 = 3.37 \text{ kcal/mol}$$

$$\Delta G_1 = 202.54$$

$$\Delta G_2 = -199.17$$

Mutant
t



$$\Delta G_{tot} = 198.90 - 189.27 = 8.63 \text{ kcal/mol}$$

$$\Delta G_1 = 198.90$$

$$\Delta G_2 = -189.27$$



6. Conclusions and Future work

- .We are able to build a reliable molecular model for HP-RNase and its mutant
- .Molecular Dynamics and distance analysis do not show specific differences
- .Alchemical Free Energy applied to the whole process is not accurate
- .We are working on computing the energetic contributions of every residue

