

HYDROLYTIC CLEAVAGE OF PHOSPHODIESTER BONDS IN RNASE A RIBONUCLEASE

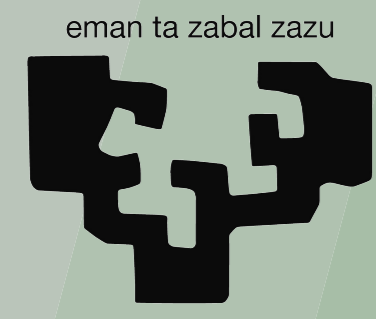
Elena Formoso and Xabier Lopez

Kimika Fakultatea, Euskal Herriko Unibertsitatea (EHU) and Donostia International Physics Center (DIPC)
P.K. 1072, 20080 Donostia-San Sebastian
Euskadi (Spain)

elena.formoso@ehu.es

Aknowledgements

The Basque Government and the Spanish Government are gratefully acknowledged for funding. The SGI/IZO-SGIker UPV/EHU (supported by Fondo Social Europeo and MCT) is gratefully acknowledged for assistance and generous allocation of computational resources.



Universidad del País Vasco Euskal Herriko Unibertsitatea

Introduction

RNase A is the predominant form of an enzyme produced by the bovine pancreas. This RNA depolymerase catalyzes the degradation of the large amount of RNA produced by microorganisms present in the forestomachs. The distributive endoribonuclease has the ability to cleave cellular RNA and thereby cause cell death. It catalyzes the hydrolysis of 3',5'-phosphodiester linkage of RNA at the 5'-ester bond on the 3' side of cytidine and uridine residues in a two-step reaction: transphosphorylation and hydrolysis.

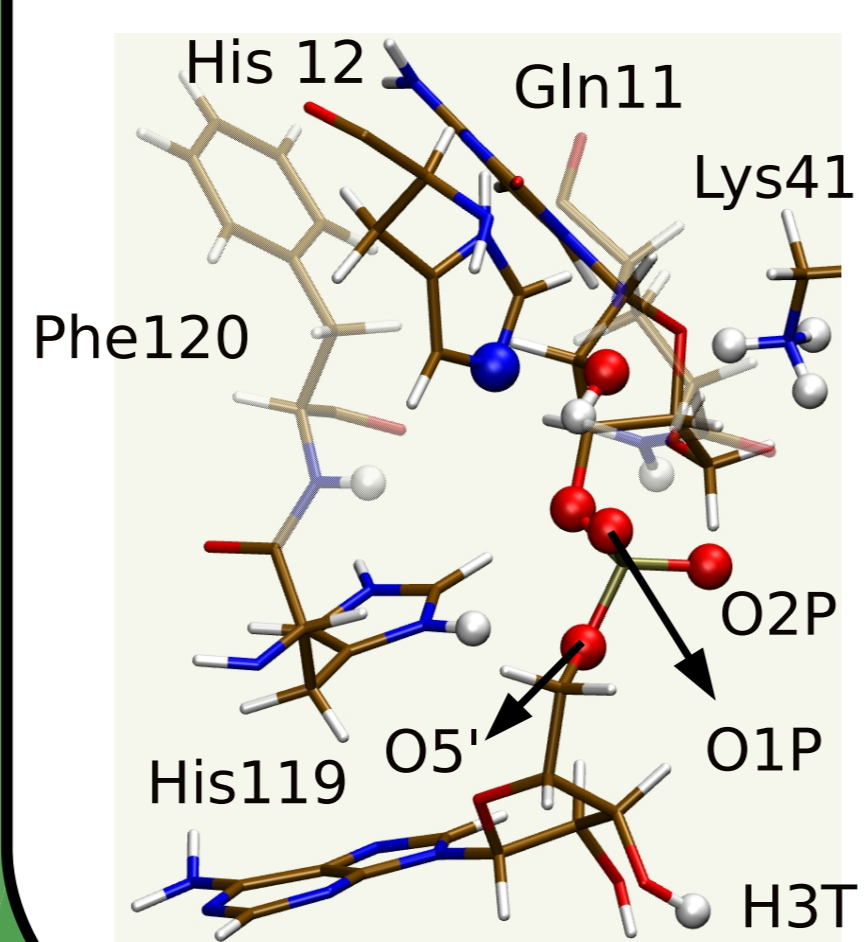
Methods

The **Periodic Boundary Conditions** Molecular dynamics method is used to **study** the structure, dynamics and **hydrogen bonds** of solvated active site of Rnase A. Simulations of the enzyme complexed with the dinucleotide substrate CpA, the transition-states analogs and thio substitutions are compared using **CHARMM c32a1** program. Rhombic dodecahedron (RHDO) water lattice-type of 70.4 Å and an ionic strength of 0.15 mol/L is applied. The entire system is equilibrated for 120ps, after equilibration 6ns simulation was performed.

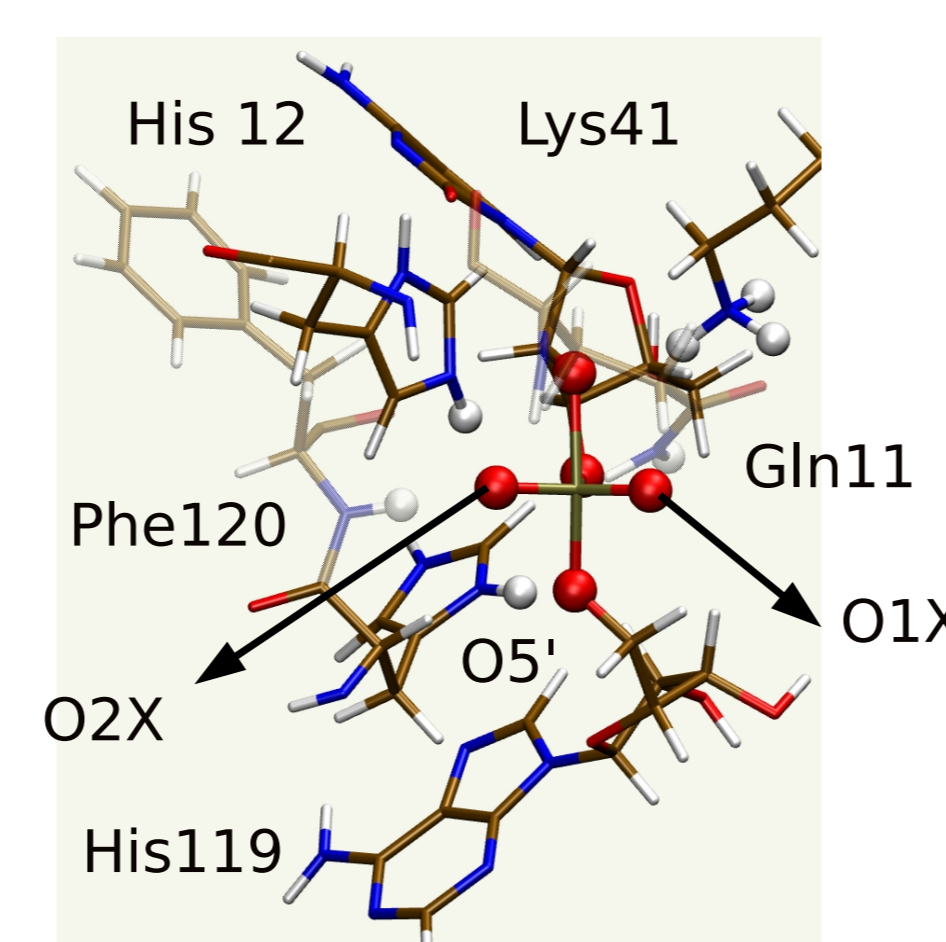
Aim of this work

Analyze the hydrogen bond pattern of the different complexes

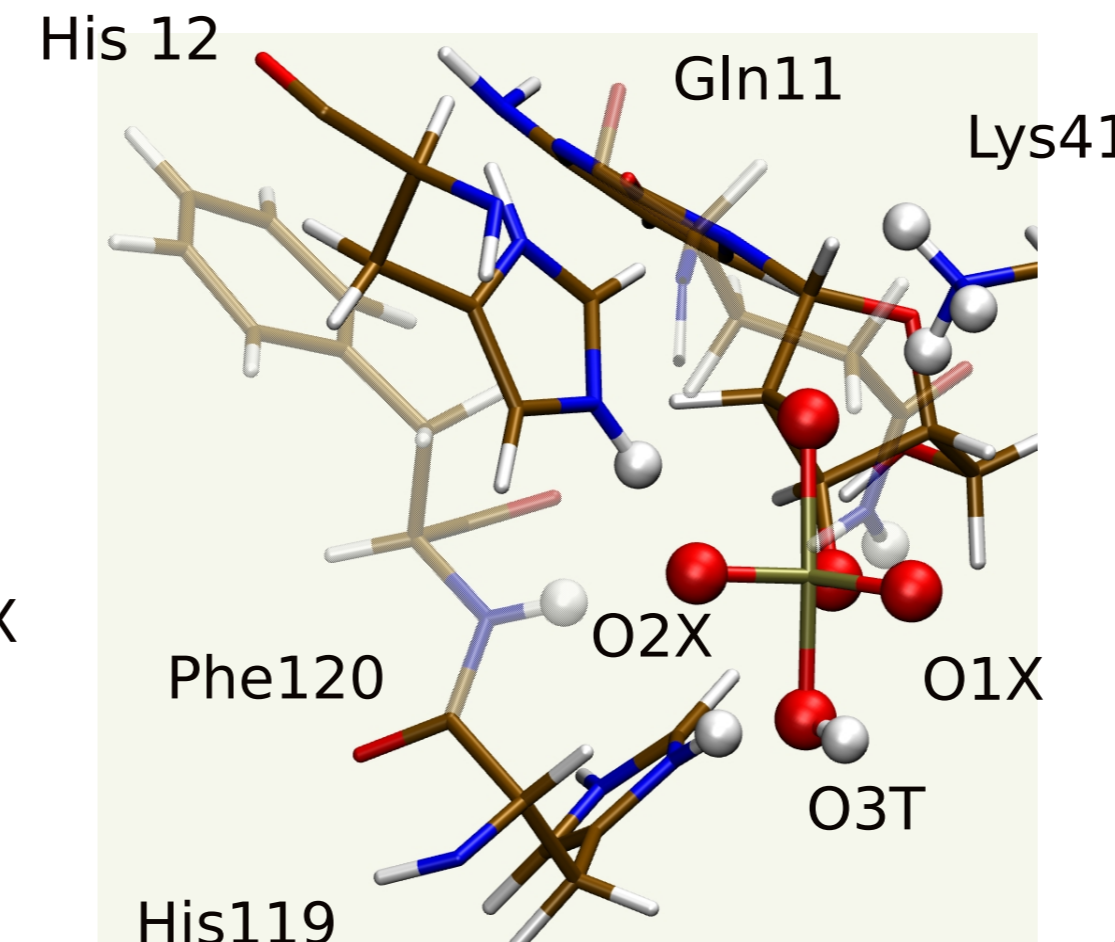
Reactant Model



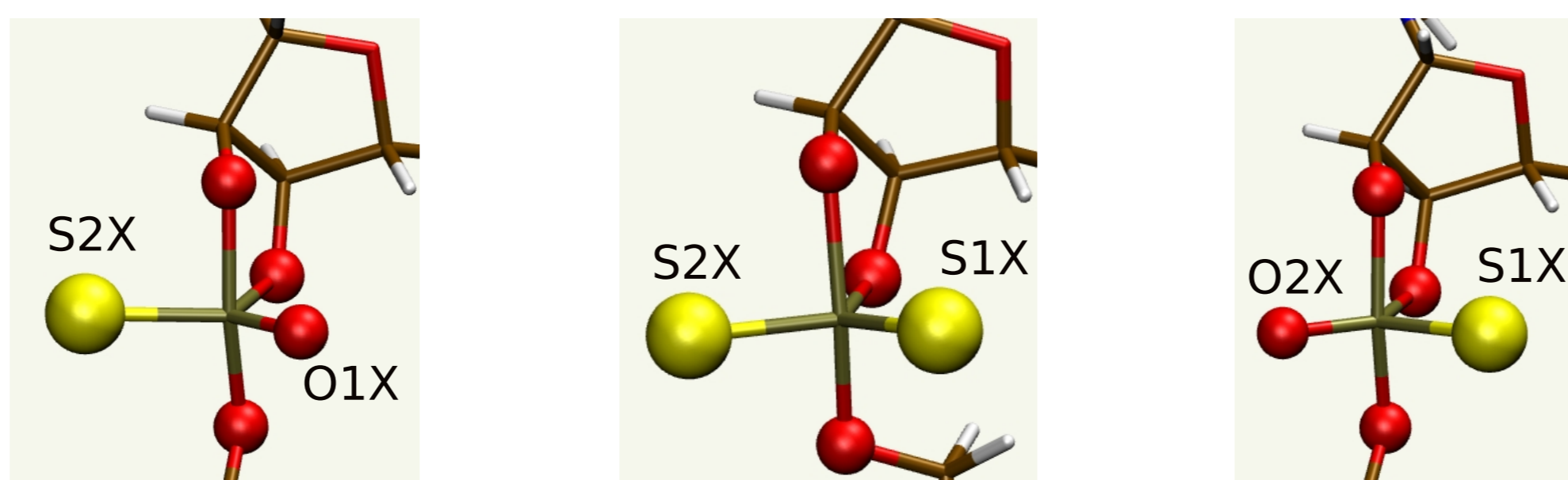
Transphosphorylation Transition State Mimic



Hydrolysis Transition State Mimic



Thio substitutions



Hydrogen bond

Distance < 2.40 Å
Angle > 120°
Lifetime ≥ 5ps

$$\text{Occupancy} = \frac{\text{Total time of this hydrogen bond}}{\text{Total time of simulation}}$$

$$\text{Average Time} = \frac{\text{Total time of this hydrogen bond}}{\text{How many times this hydrogen bond is form}}$$

Results and Discussion

- His119**
 - Occupancy of nearly 1 for O5' or O3T
 - Time average
 - O5' > 150ps except reactant model
 - O3T ≤ 120ps
- Phe120**
 - Occupancy
 - > 0.7 for O1P and O2X
 - < 0.1 for S2X
 - Time average
 - < 70ps for O2X
 - < 15ps for S2X
- His12**
 - Occupancy
 - ~ 1 for O2X
 - < 0.5 for S2X
 - * Transphosphorylation TS mimic: reduced from 0.4 to ~ 0.13 when the thio substitutions are done
 - * Hydrolysis TS mimic: increment from 0.15 to ~ 0.3 when S2X thio substitution is done
 - Time average
 - > 200ps for O2X
 - ~ 20ps for S2X
 - between 20 and 10ps for O2'
- Lys41**
 - Occupancy for O2' and equatorial oxygen
 - Reactant and TS mimics → O2' > equatorial oxygen
 - S1X and dithio substitutions → O2' nearly the only hydrogen bond
 - S2X thio substitution → O1X > O2'

