# 第一原理ハイブリッド分子動力学計算によるアミノアシル tRNA 合成酵素の触媒反応機構の解析

# Catalytic mechanisms of enzymatic reactions of protein-RNA complexes investigated by hybrid molecular dynamics simulations


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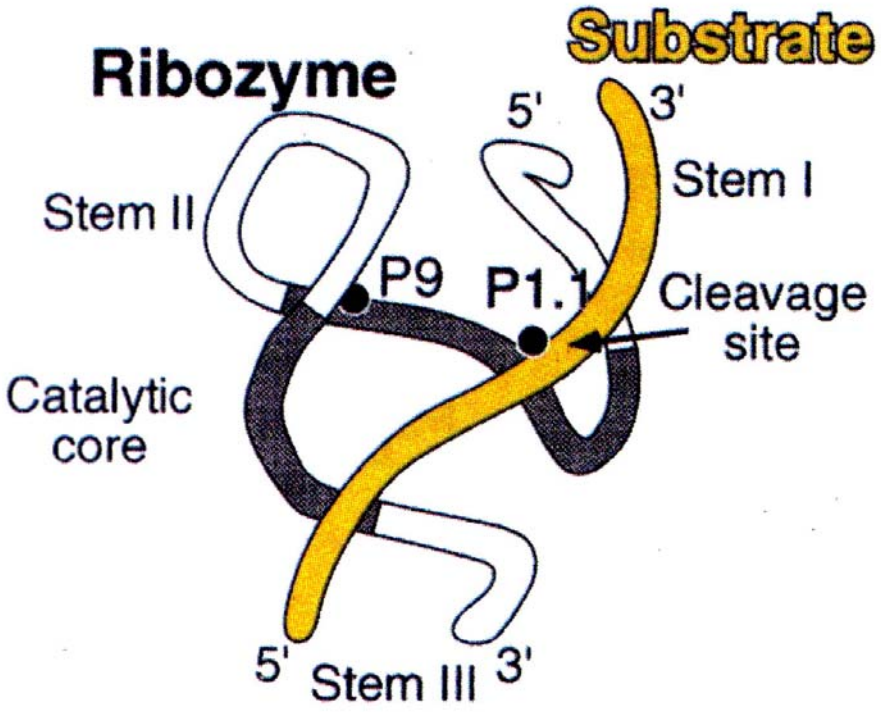
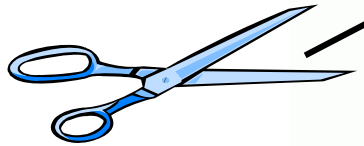
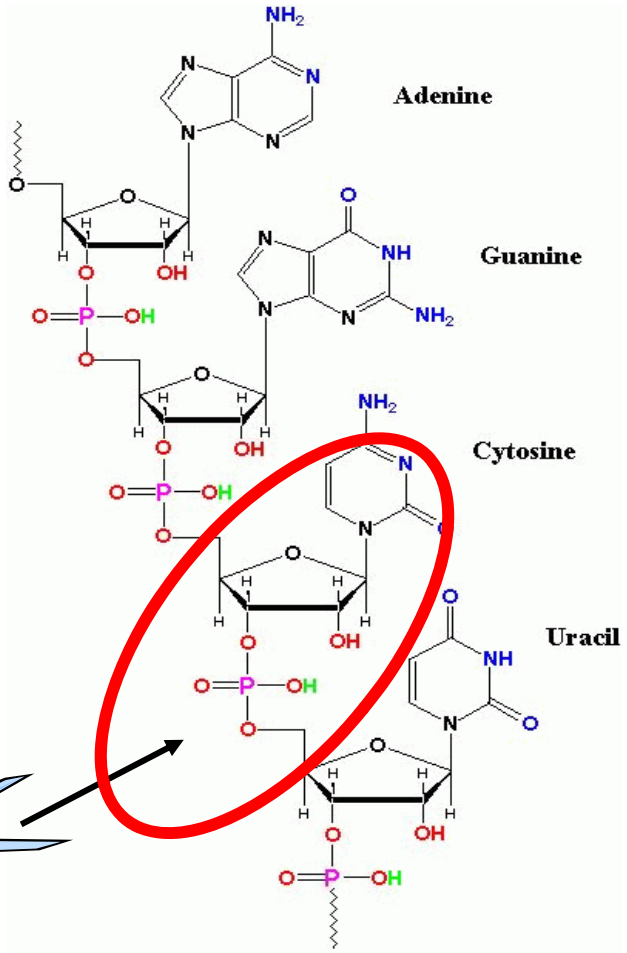
# Reaction mechanisms of biomacromolecules: Hammerhead Ribozyme (RNA enzyme)





# Ribozymes in biological and medical sciences

**1) Biological function**

Contribution to transfer of genetic information
from DNA to protein

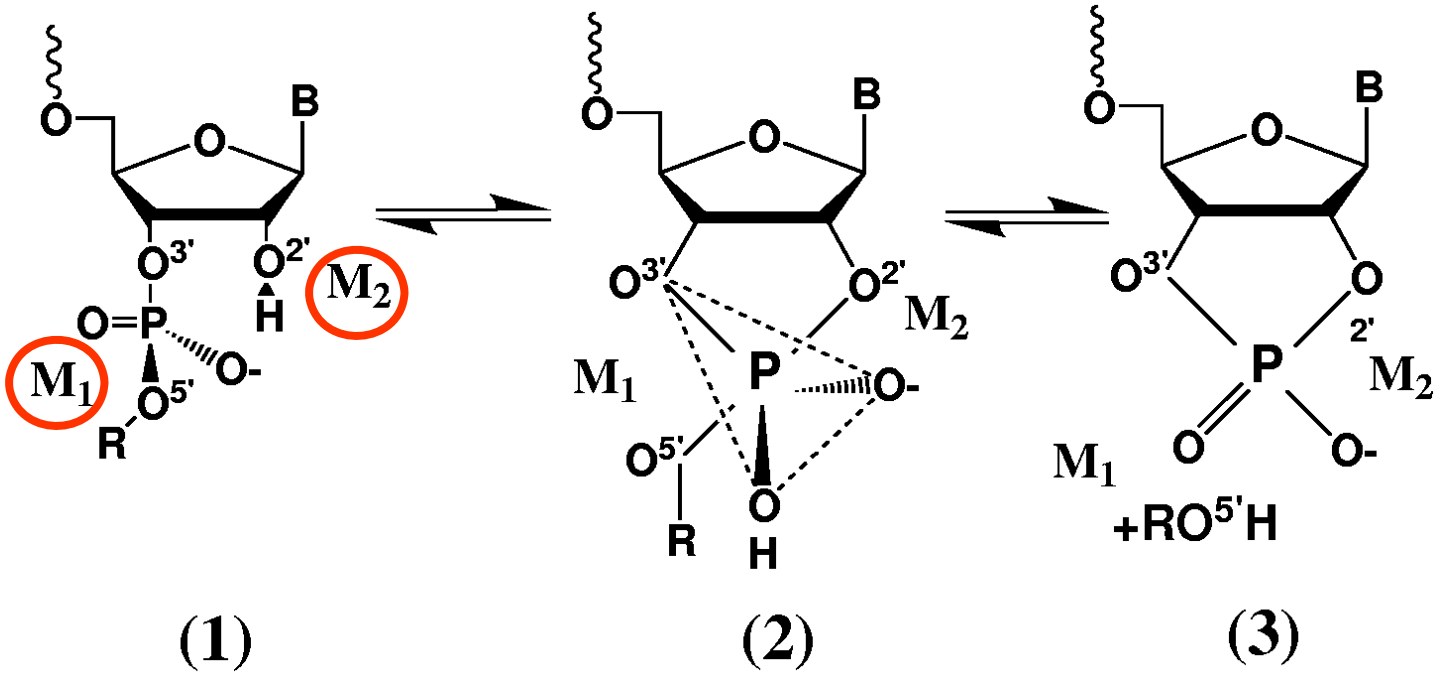
**2) Evolution of organisms**

| | primordial organism (in the RNA world) | present organism (in the RNP world) |
|---|---|---|
| gene | RNA | DNA |
| enzyme | RNA | protein |

**3) Medical application**

Inhibition of expression of genes, such as oncogene
→ Gene therapy

# Ribozymes reaction catalyzed by divalent metal ions




Fundamental questions:

- How many cations (one or two) are involved in the reaction ?
- Catalytic mechanisms ?

A. M. Pyle, *Science* **261**, 709 (1993)

# Computational Details for the QMMM

- Quantum subsystem:

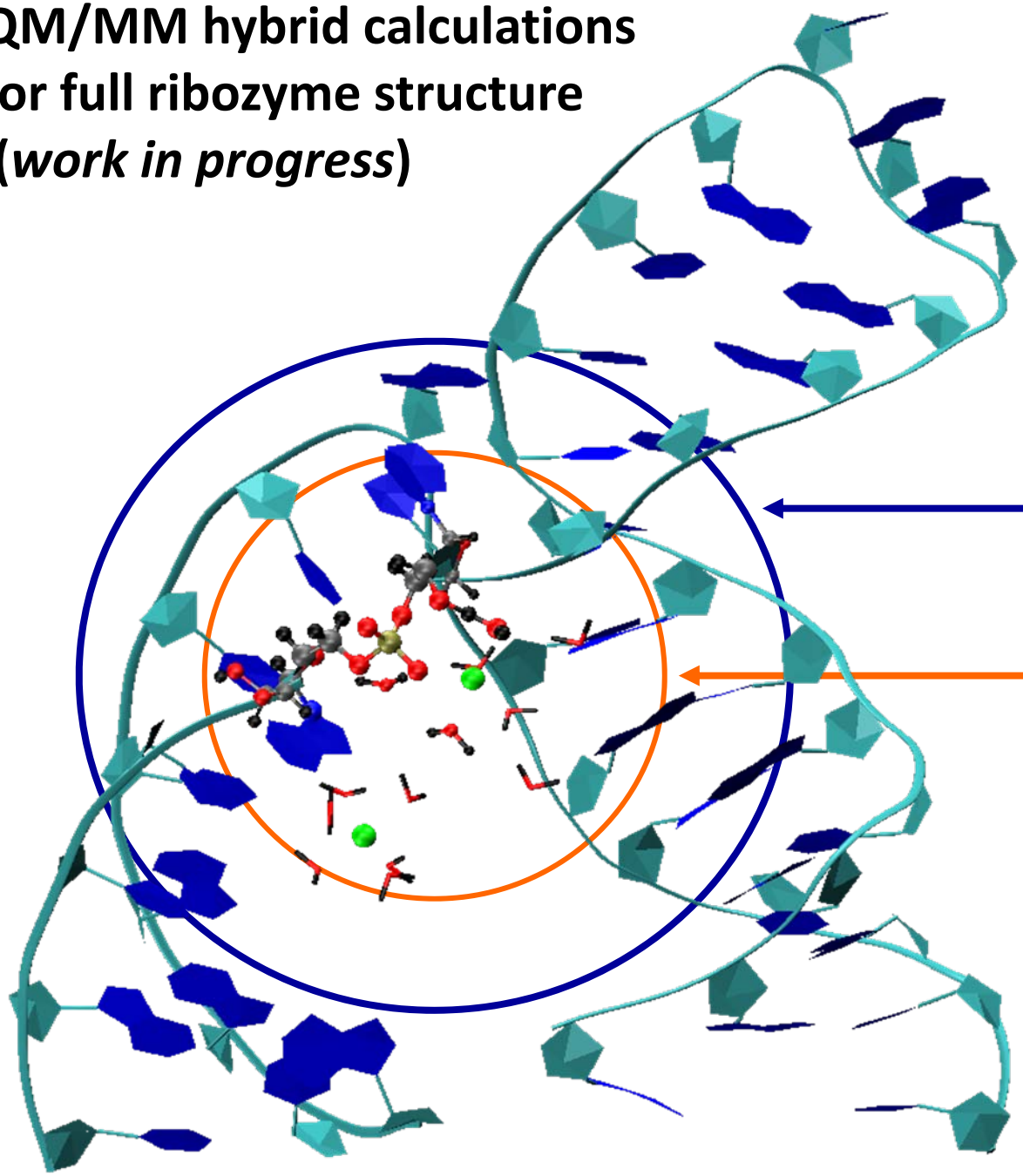
  - Car-Parrinello molecular dynamics with HCTH DFT/LSD
  - Norm-conserving Troullier-Martins pseudopotentials
  - Valence wave functions expanded in PW with cut-off of 70 Ry
  - 90 atoms including 14 $H_2O$ molecules + $OH^-$
  - Metadynamics for reaction paths sampling
  - Temperature control: Nose'-Hoover thermostat chain

- Whole system:

  - 44963 atoms (entire hammerhead ribozyme fully hydrated)
  - Amber classical force field
  - RESP coupling for the MM part close to the QM subsystem

  $r_{cut}$(NN) = 10 a.u.  $r_{cut}$(Mix) = 15 a.u.  $r_{cut}$(RESP) = 25 a.u.

# QM/MM hybrid calculations for full ribozyme structure (*work in progress*)



The interacting QM/MM part of the system

**D-RESP**

$r_1 < r < r_2$

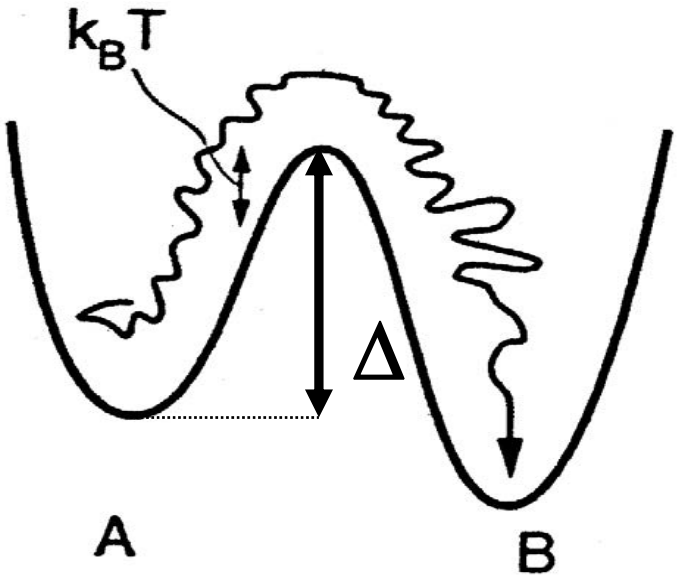
**QM**

$r < r_1$

# From reactants A to products B: 山登

- A general chemical reaction starts from reactants A and goes into products B
- The system spends most of the time either in A and in B
- …but in between, for a short time, a barrier is overcome and **atomic** and **electronic** modifications occur
- Time scale:

$$\tau \sim \tau_{mol} e^{\frac{F^*}{k_B T}}$$




$$F^* \sim \Delta$$

# Sampling the reaction path via metadynamics

1) The atomic and electronic configuration of our initial system is given by a set of variables

$$\{\mathbf{R}_1,\ldots,\mathbf{R}_N\}_{t=0} \oplus \{\psi_1,\ldots,\psi_M\}$$

2) …and we assume that some known functions of a subset of them (collective variables) are necessary and sufficient to describe the process we are interested in

$$s_\alpha(\mathbf{R}_I;\psi_i) \qquad \alpha = 1,\ldots,n < N,M$$

3) …so that the FES is a function of these changing variables

$$F(\mathbf{s}) \qquad \mathbf{s} \equiv \mathbf{s}(t) = \{s_\alpha(t)\}_{\alpha=1,\ldots,n}$$

# Metadynamics Collective variable(s) ?

- Distances
- Angles (bending, torsion, out-of-plane, etc.)
- Coordination numbers
- Spin density
- Local electric fields
- number of n-fold rings
- Lattice vectors
- Energy
- etc…

# Metadynamics in few words:

- ***Artificial*** dynamics in the space of a few collective variables [1]
- The CPMD dynamics is biased by a history-dependent potential constructed as a sum of Gaussians [2].
- The history dependent potential compensates the underlying free energy surface [3,4].

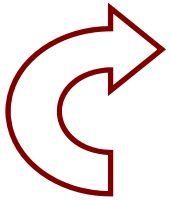

[1] I. Kevrekidis et al, *Comput. Chem. Eng.* (2002)
[2] T. Huber et al, *J. Comput.* (1994)
[3] F. Wang and D. Landau, *Phys. Rev. Lett.* (2001)
[4] E. Darve and A. Pohorille, *J. Chem. Phys.* (2001)


# What is it used for ?

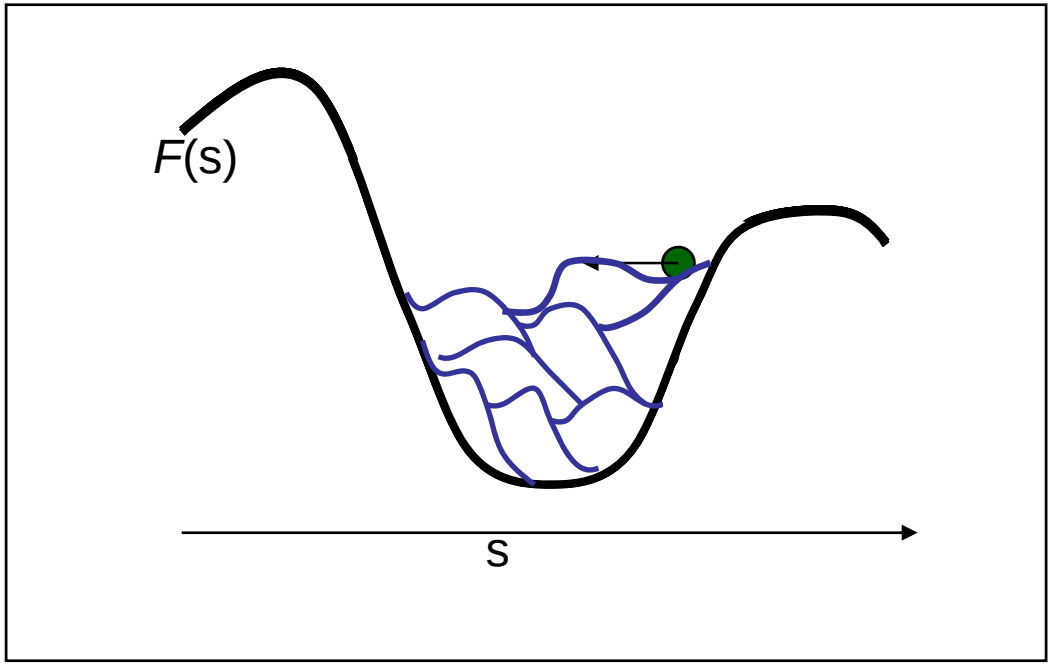
- For reconstructing $F(\mathbf{s})$ in many dimensions as a function of one or more collective variables.
- Used as a tool for escaping local (free energy) minima and exploring the free energy in the selected phase space


M. Iannuzzi, A. Laio and M. Parrinello, *Phys. Rev. Lett.* **2003**, *90*, 238302
A. Laio and M. Parrinello, *Proc. Nat. Ac. Sci.* **2002**, *99*, 12562

# Metadynamics: how does it work ?



- Put a “small” Gaussian
- The dynamics brings you to the closest local minimum of *F*(s) plus the sum of all the Gaussians



# How to plug all this in CPMD ?

Fictitious kinetic energy

$$\mathcal{L} = \mathcal{L}_{CP} + \sum_{\alpha} \frac{1}{2} M_{\alpha} \dot{s}_{\alpha}^2 - \sum_{\alpha} \frac{1}{2} k_{\alpha} \left( S_{\alpha}(\mathbf{R}) - s_{\alpha} \right)^2 - V(\mathbf{s}, t)$$

Restrain potential: coupling fast and slow variables

$\sqrt{(k_\alpha/M_\alpha)} \ll \omega_I$

$$\frac{\partial \mathcal{F}}{\partial s_{\alpha}} \simeq \langle k_{\alpha} (S_{\alpha}(\mathbf{R}) - s_{\alpha}) \rangle$$

**History-dependent potential**

# Analytic form of $V(\mathbf{s},t)$ in CPMD:

$$V(\mathbf{s},t)=\sum_{t_i<t} W_i \exp\left(-\frac{1}{2}\frac{(\mathbf{s}-\mathbf{s}_i)^2}{(\Delta s^{\perp})^2}\right)\exp\left[-\frac{1}{2}\frac{\left((\mathbf{s}_{i+1}-\mathbf{s}_i)\cdot(\mathbf{s}-\mathbf{s}_i)\right)^2}{(\Delta s_i^{\parallel})^4}\right]$$

and the discrete time step $\Delta t$ must be such that

CPMD time step → $\Delta t^{CPMD} << \Delta t << \omega_{\vec{s}_\alpha}^{-1}$ ← Highest oscillation frequency of $s_\alpha$

Free Energy ⟹ $\lim_{t\to\infty} V(\mathbf{s},t) = -F(\mathbf{s}) + const.$

# Double-metal-ion reaction via metadynamics in the presence of an $OH^-$ close to $O^{2'}$-H

(see e.g. S. Sawata et al. *JACS* **117**, 2357 (1995))

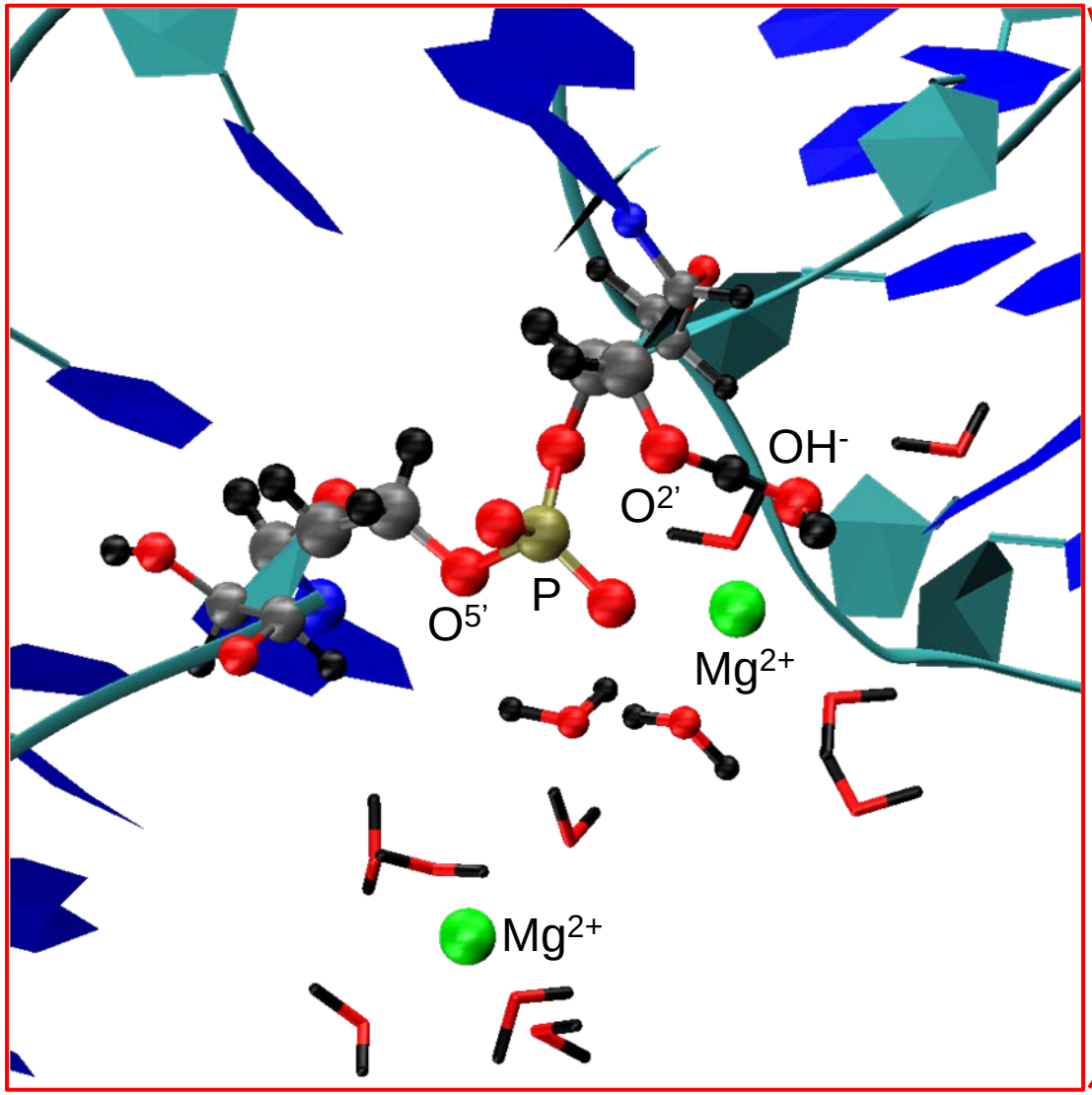
- Collective variables:

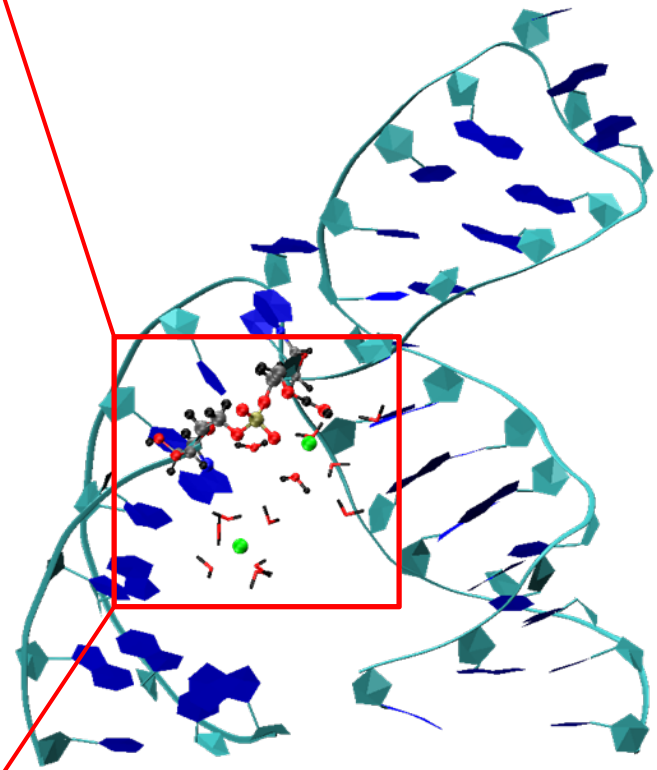
  $s_1(H^{2'}\text{-}O)$ = distance of the (deprotonating) $H^{2'}$ proton and oxygen atom of the hydroxyl anion $OH^-$

  $s_1(|P\text{-}O^{2'}|\text{-}|P\text{-}O^{5'}|)$ = difference of distances describing P-O bond breaking/formation

- $V(s_\alpha,t)$ updated every 200 CPMD steps (0.02 ps)
- Spherical Gaussian functions with amplitudes sampled in the range 0.15 kcal/mol < $W_i$ < 2.00 kcal/mol

Hybrid QM/MM metadynamics with $OH^-$



# QM/MM hybrid calculation of catalytic reaction of ribozyme

Novel mechanism evidenced:

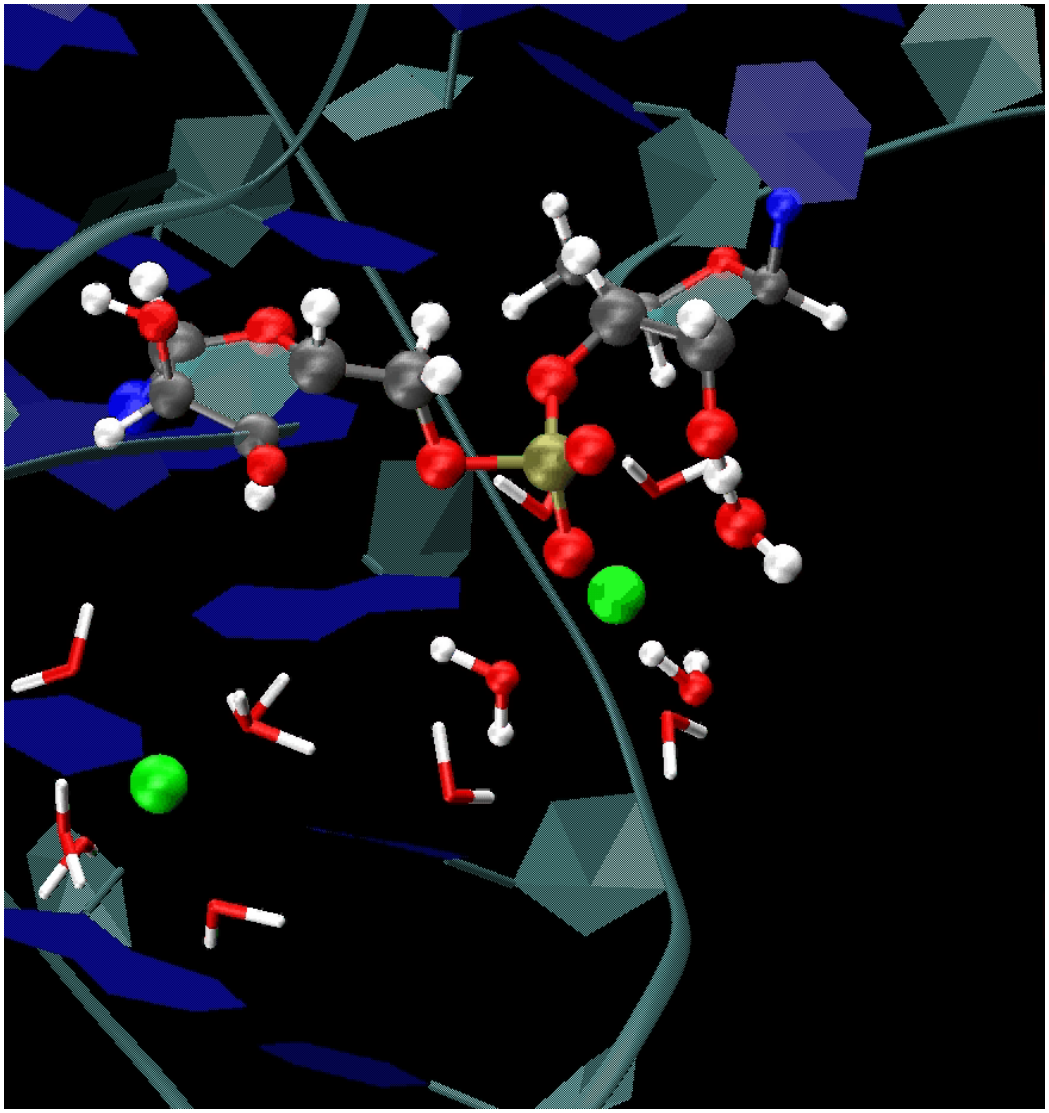
- One single $Mg^{2+}$ cation can play a double role, as in two-ions mechanism.

-Activation barrier

$\Delta F = 29.6$ kcal/mol

(Experiment: 28 kcal/mol)

- The reaction mechanism is very similar to the double-ion mechanism



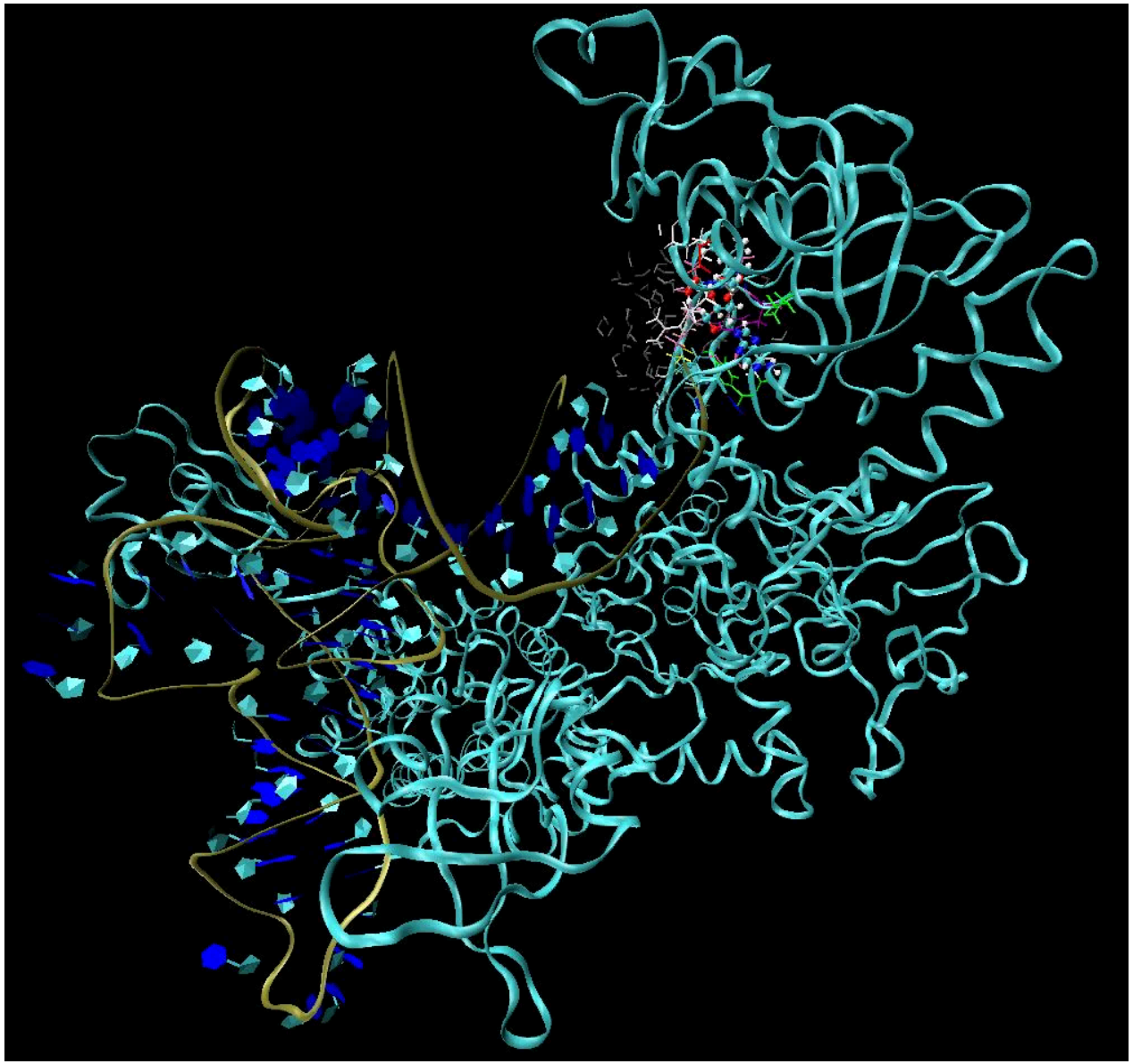
- Leucyl-tRNA synthetase (LeuRS) is one of the 20 enzymes aminoacyl-tRNA synthetases family
- It is responsible for aminoacylating the aliphatic amino acid leucine and tRNA editing
- LeuRS recognizes the adenosine residue at position A73 of tRNA, known as discriminator
- But how LeuRS recognizes the discriminator A73 and and how the tRNA editing reaction occurs is unknown

See, for instance

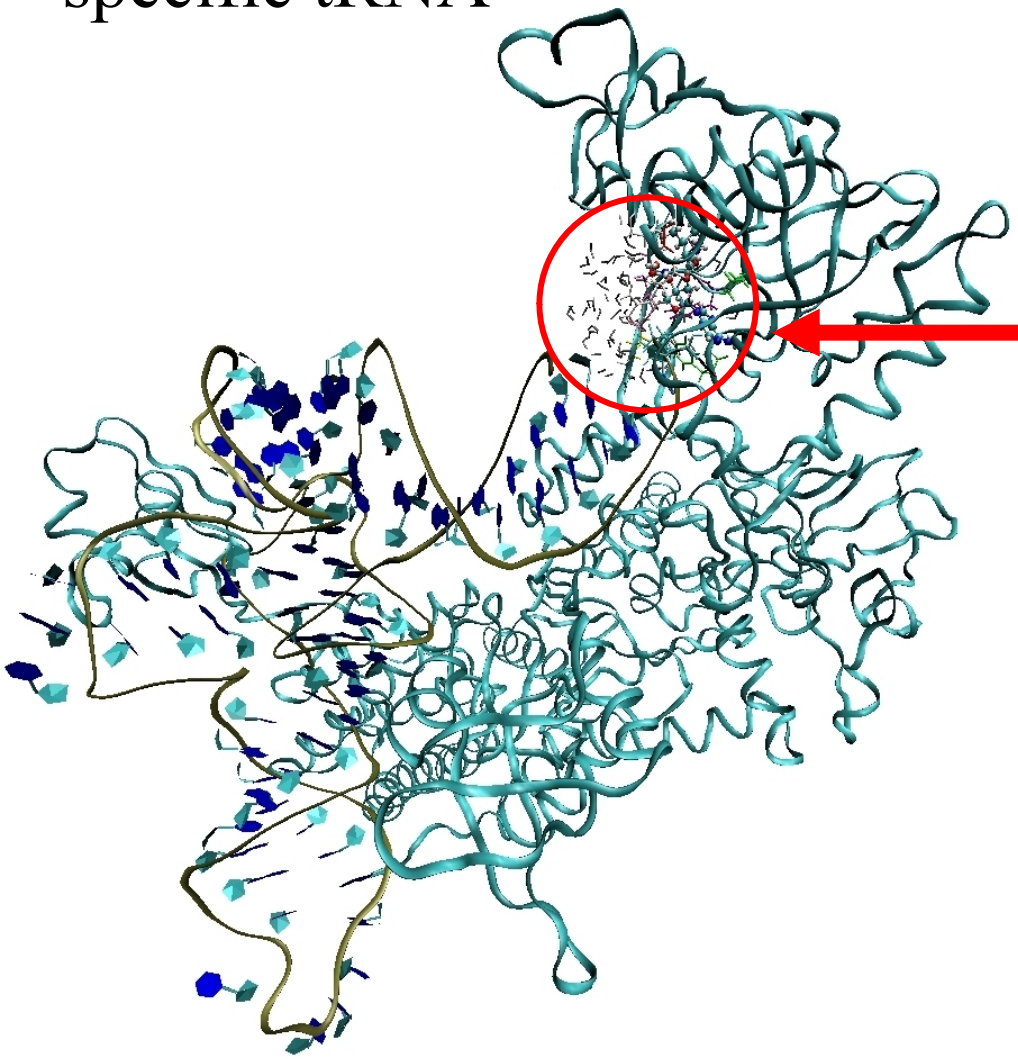
R. Fukunaga and S. Yokoyama, *Nature Struct. Mol. Biol*. **12,** 915 (2005)

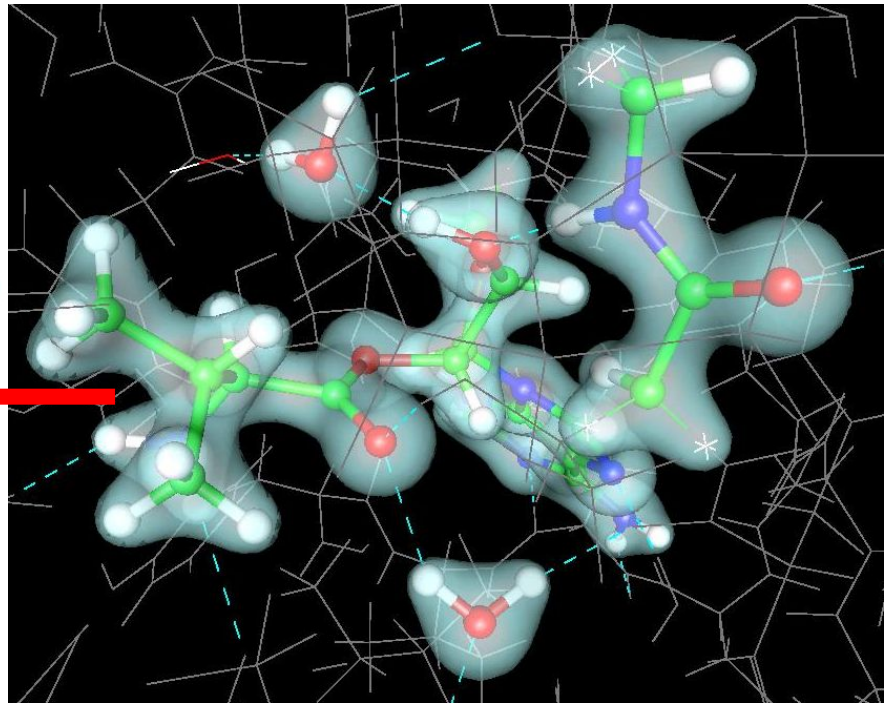
M. Xu et al. *J. Biol. Chem.* **277**, 41590 (2002)

QM/MM simulation of the complex of LeuRS with its specific tRNA: One of the active sites in the editing reaction



# QM/MM Simulation of the Complex of LeuRS and its specific tRNA





One of the Active Sites in LeuRS (Editing Reaction)

## System size analyzed:

MM: 165750 atoms

QM: 63 atoms + 5 capping H atoms

LSDA and HCTH functional

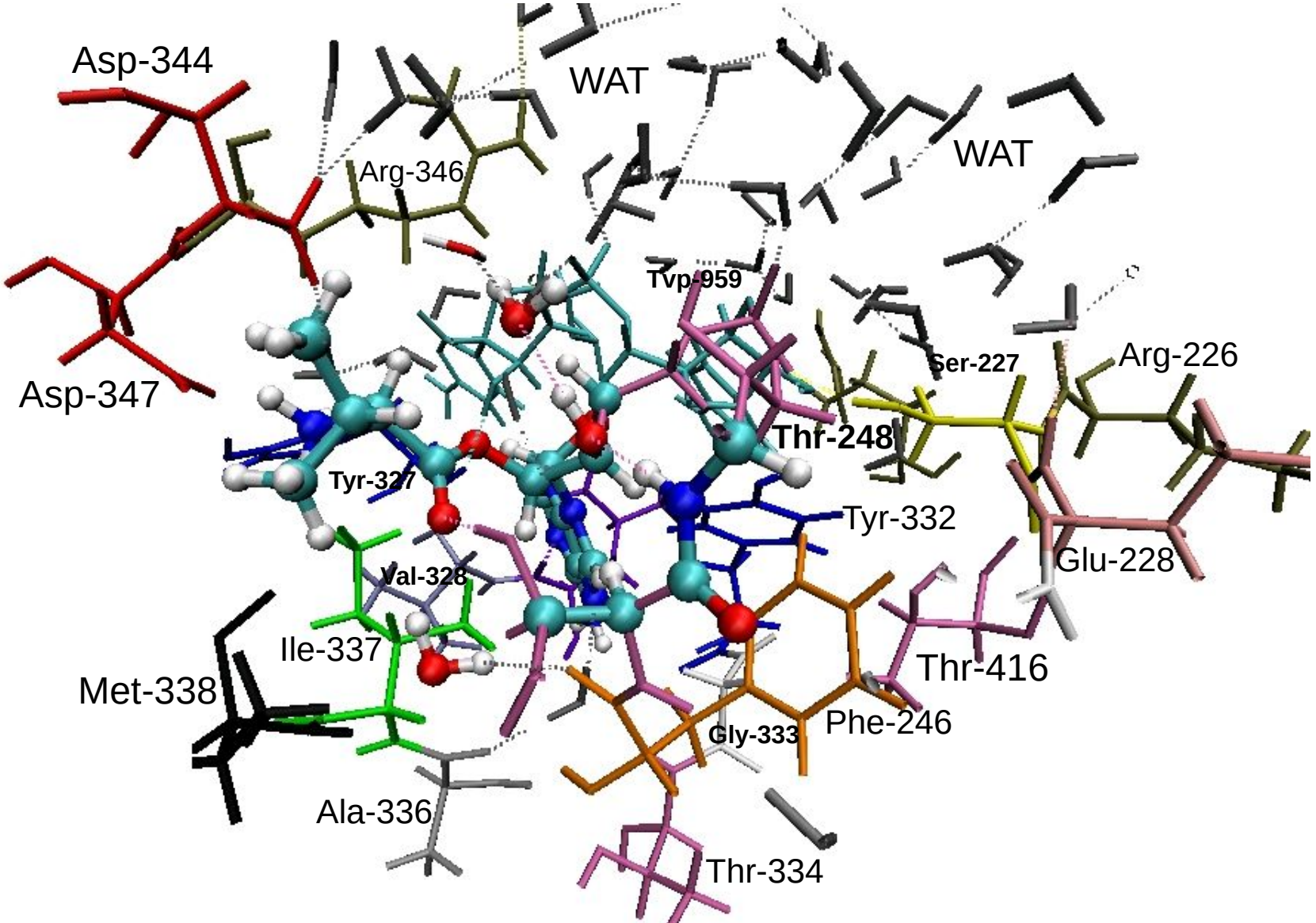
$E^{cut}$=70 Ry  176 $e^-$ (Q = +1)

164759 PWs

QM Cell = 17x15x21 Å$^3$

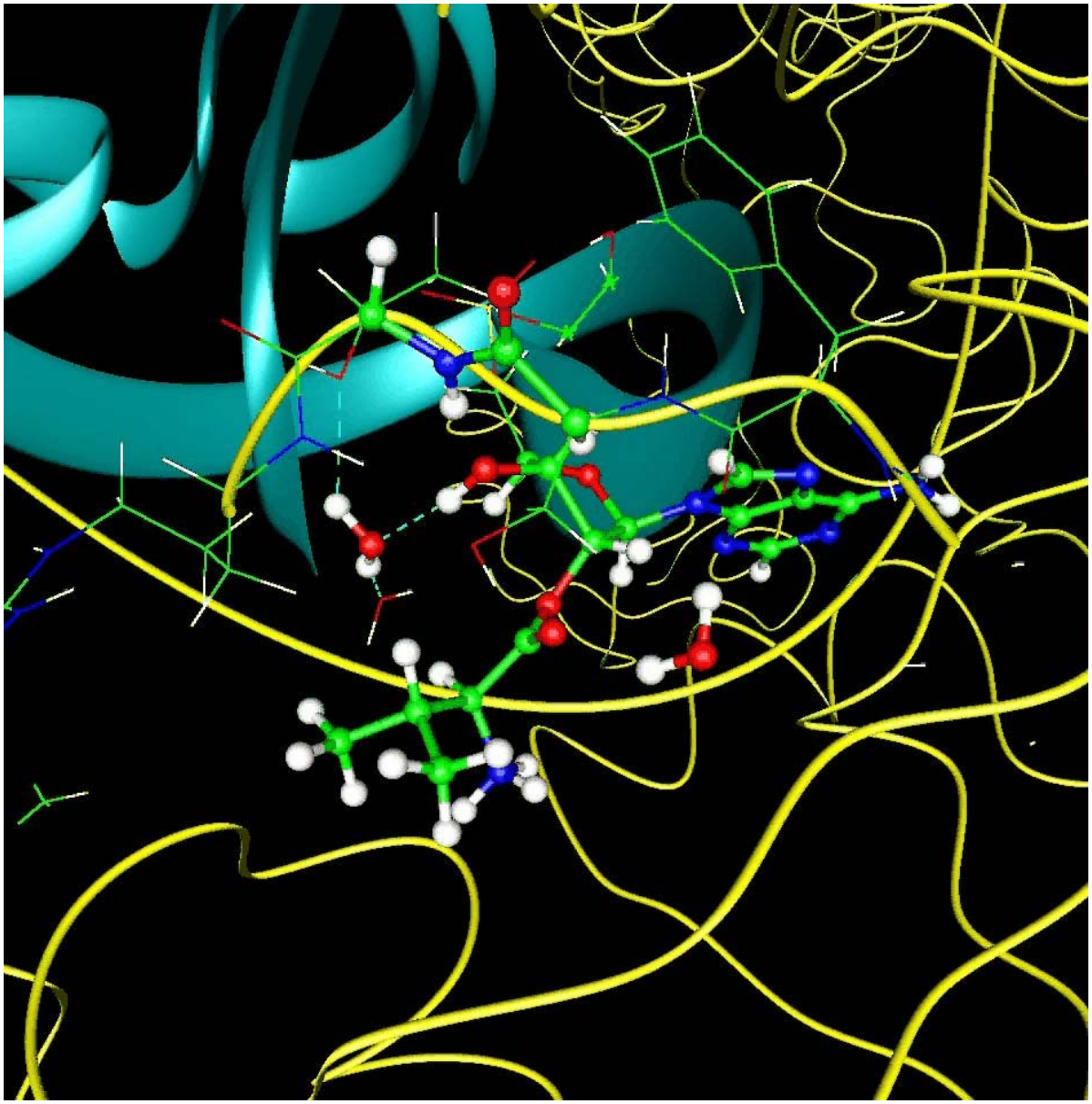
$N_x x N_y x N_z$ = 180x144x216

MM residues and solvating water molecules included in a 15 Å shell around the QM subsystem represented by balls and thick sticks

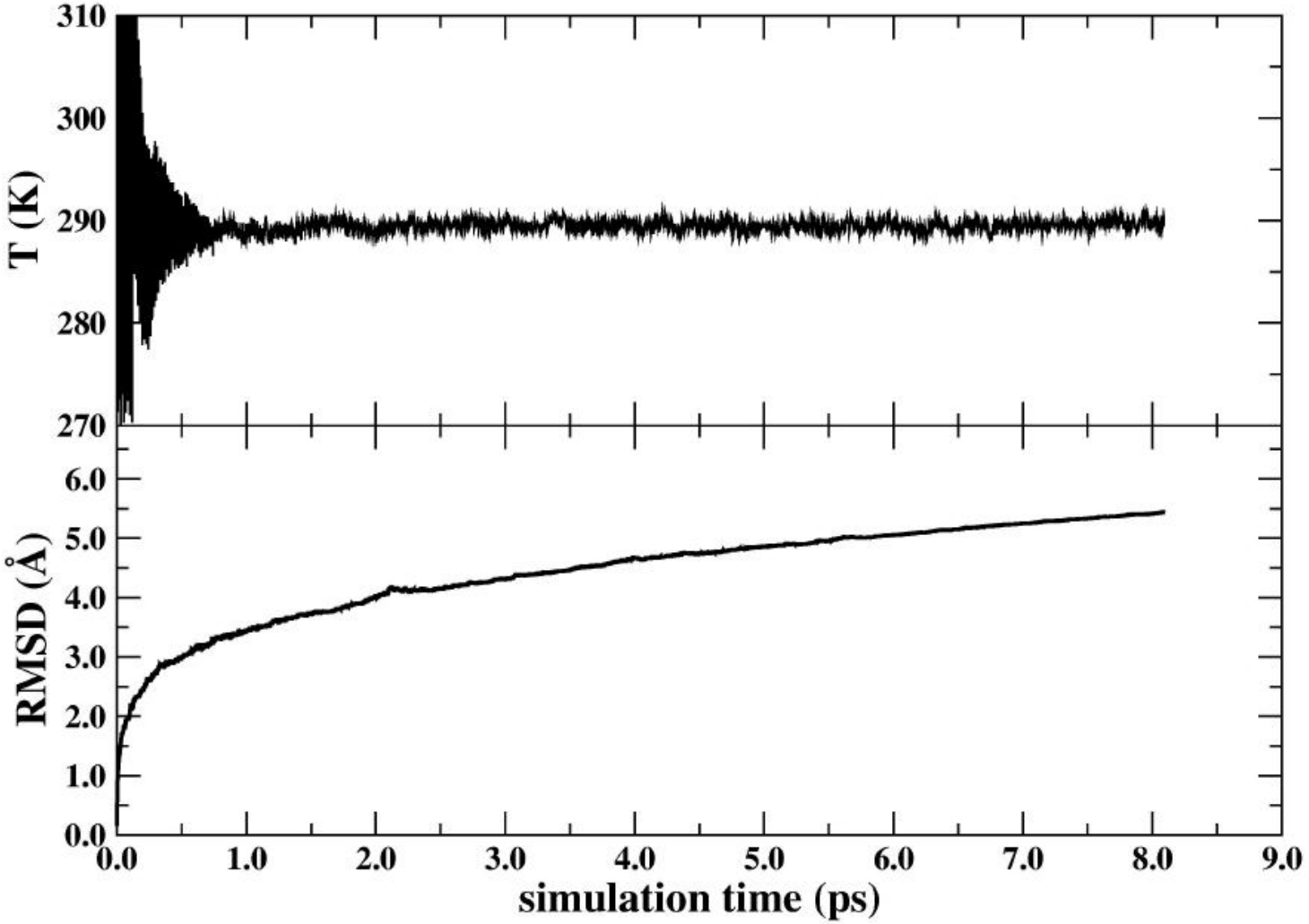



QM atoms: red = **O**, cyan = **C**, blue = **N**, white = **H**

# QM/MM equilibration LeuRS – tRNA complex

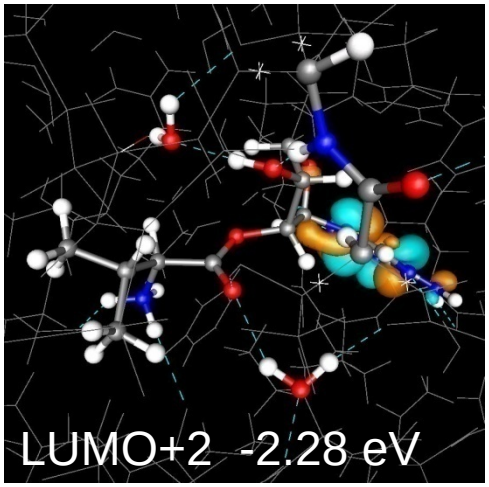


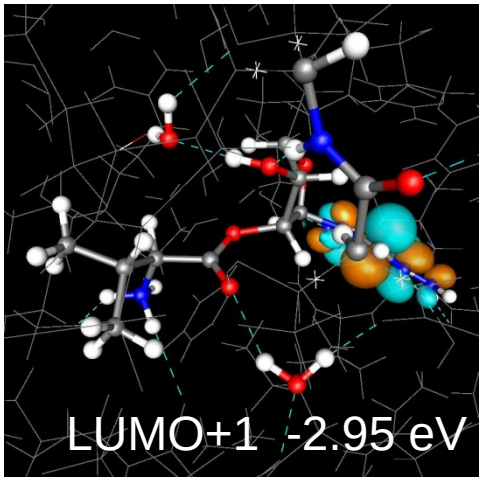
# Equilibration (so far !) via QM/MM CPMD

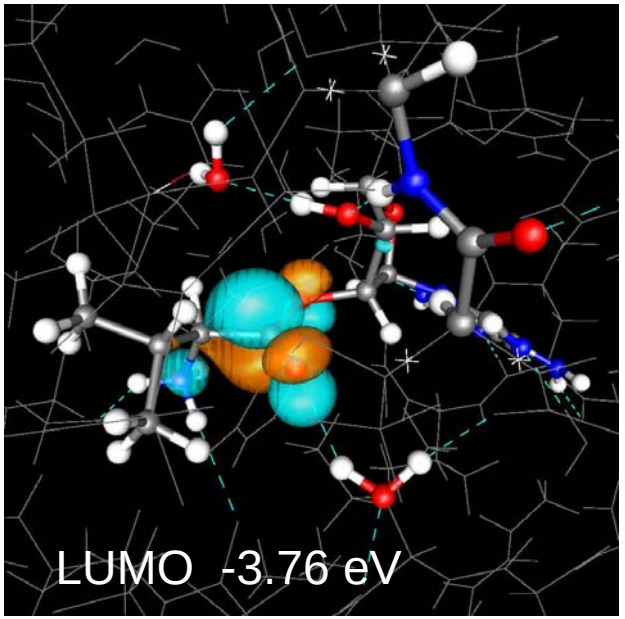


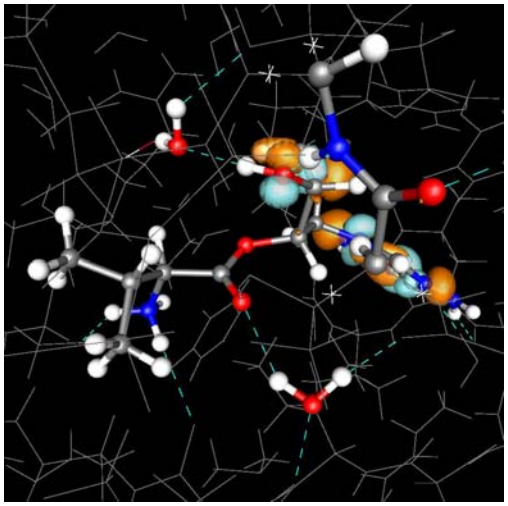
Initial electronic structure:

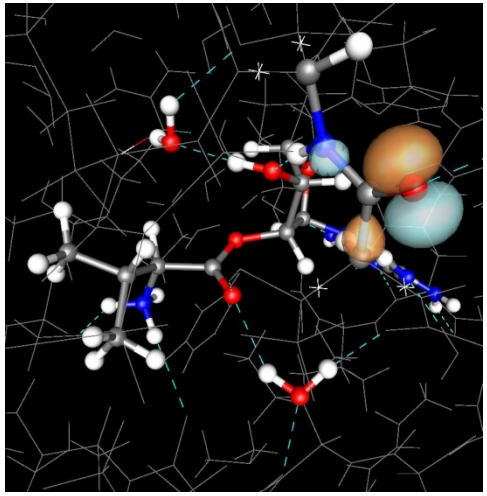
$E_{gap}$ = 2.85 eV, Isosurface= ±0.05 1/Å$^{3/2}$

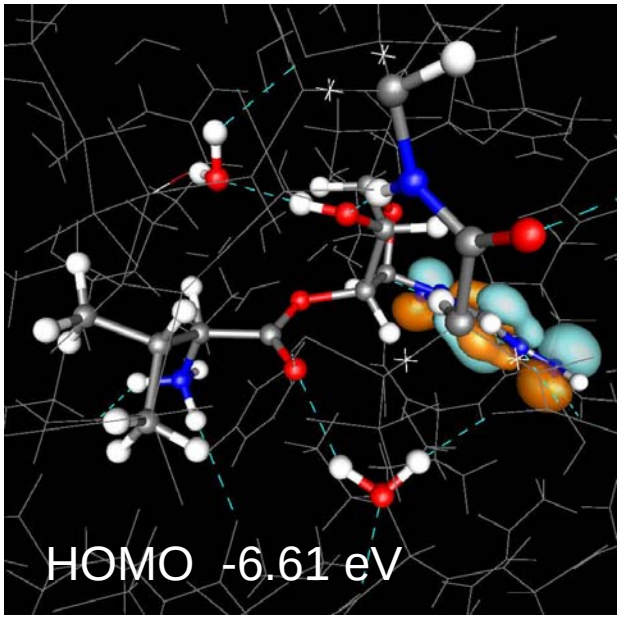


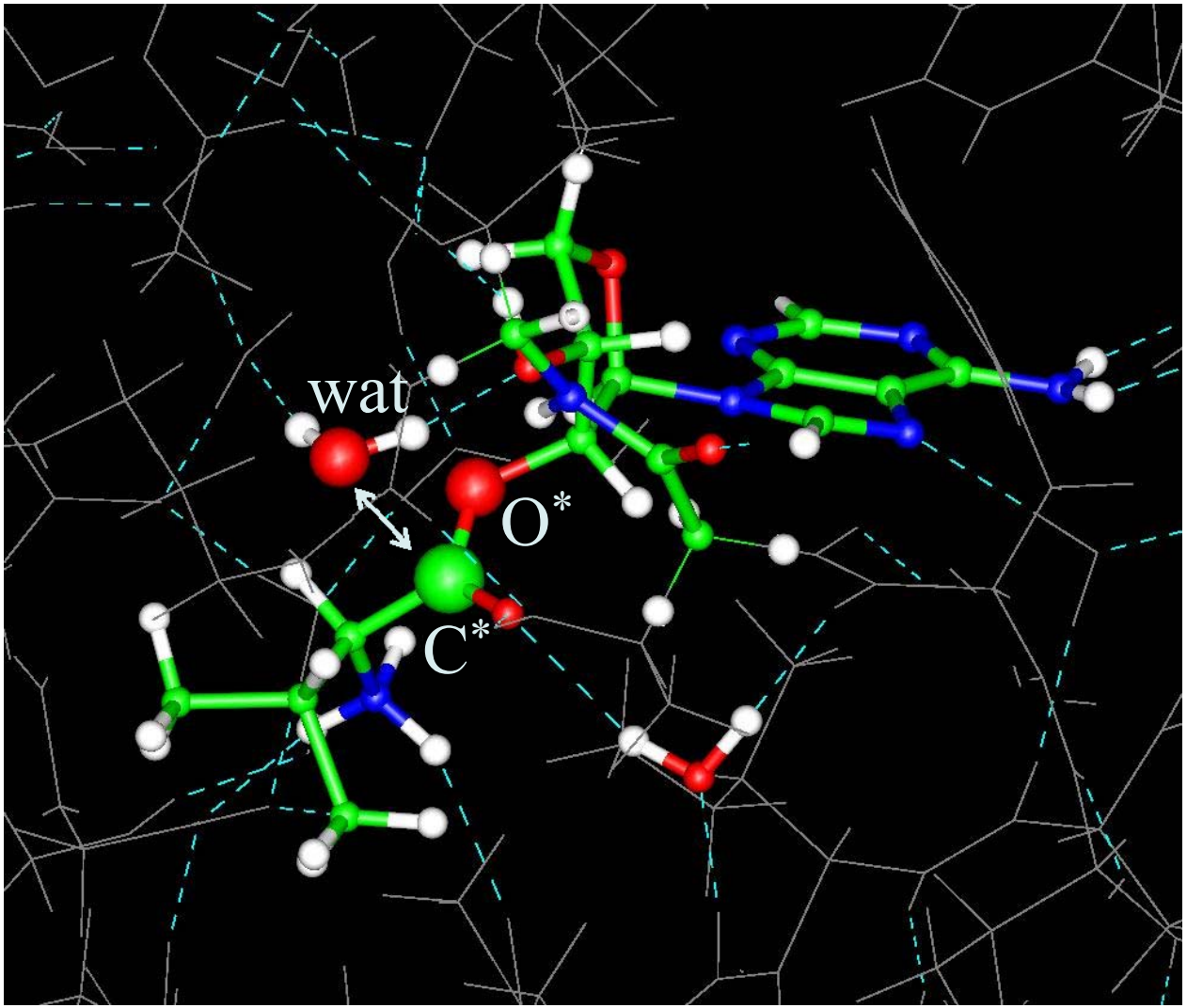







# Reaction coordinates $s_\alpha(t)$ sampled via metadynamics:




Simulation 1

$s_1 = |C^*\text{-}O^{wat}|$

$s_2 = |O^*\text{-}H^{wat}{}_1|$

Simulation 2

$s_1 = |C^*\text{-}O^{wat}|$

$s_2 = |O^*\text{-}H^{wat}{}_2|$

# Conclusions (so far):

- Hammerhead ribozyme reaction mechanism elucidated and activation barrier computed
- Catalytic role of $Mg^{2+}$ and $OH^-$ understood
- LeuRS – tRNA reactive complex obtained in the equilibration stage
- Comprehension of the electronic structure of the LeuRS - tRNA-$H_2O$ reactive system

Related recent publications:


M. Boero, T. Ikeda, E. Ito and K. Terakura, *J. Am. Chem. Soc*. **128**, 16798 (2006)
F. L. Gervasio, M. Boero and M. Parrinello, *Angew. Chem. Int. Ed*. **45**, 5606 (2006)
M. Boero, F. L. Gervasio and M. Parrinello, *Mol. Simul*. **33**, 57 (2007)
M. Boero, J. M. Park, Y. Hagiwara and M. Tateno, *J. Phys. Cond. Mat*. **19**, 365217 (2007)
T. Ikeda, M. Boero and K. Terakura, *J. Chem. Phys.* **127**, 074503 (2007)
K. Kamiya, M. Boero, M. Tateno, K. Shiraishi and A. Oshiyama, *J. Am. Chem. Soc*. **129**, 9663 (2007)