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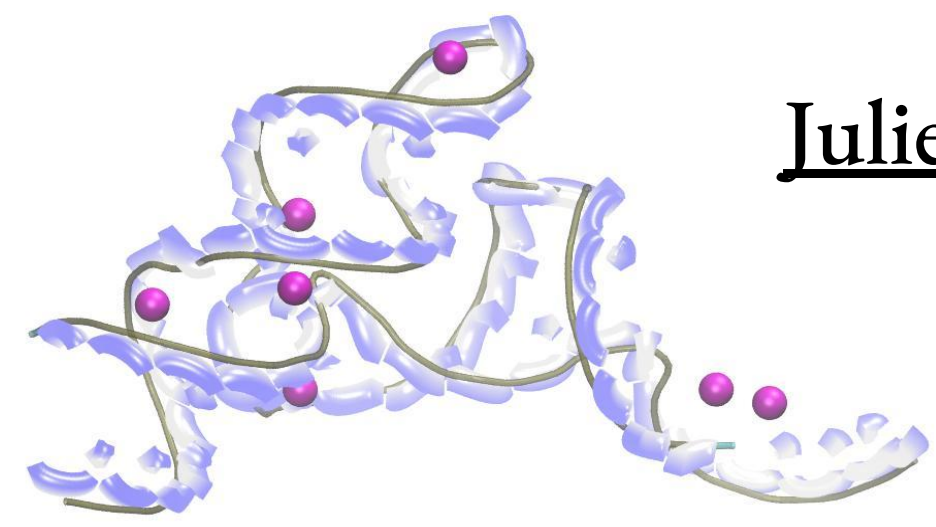
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Exploring the Role of Divalent Cations in the Hammerhead Ribozyme Mechanism with Molecular Simulations



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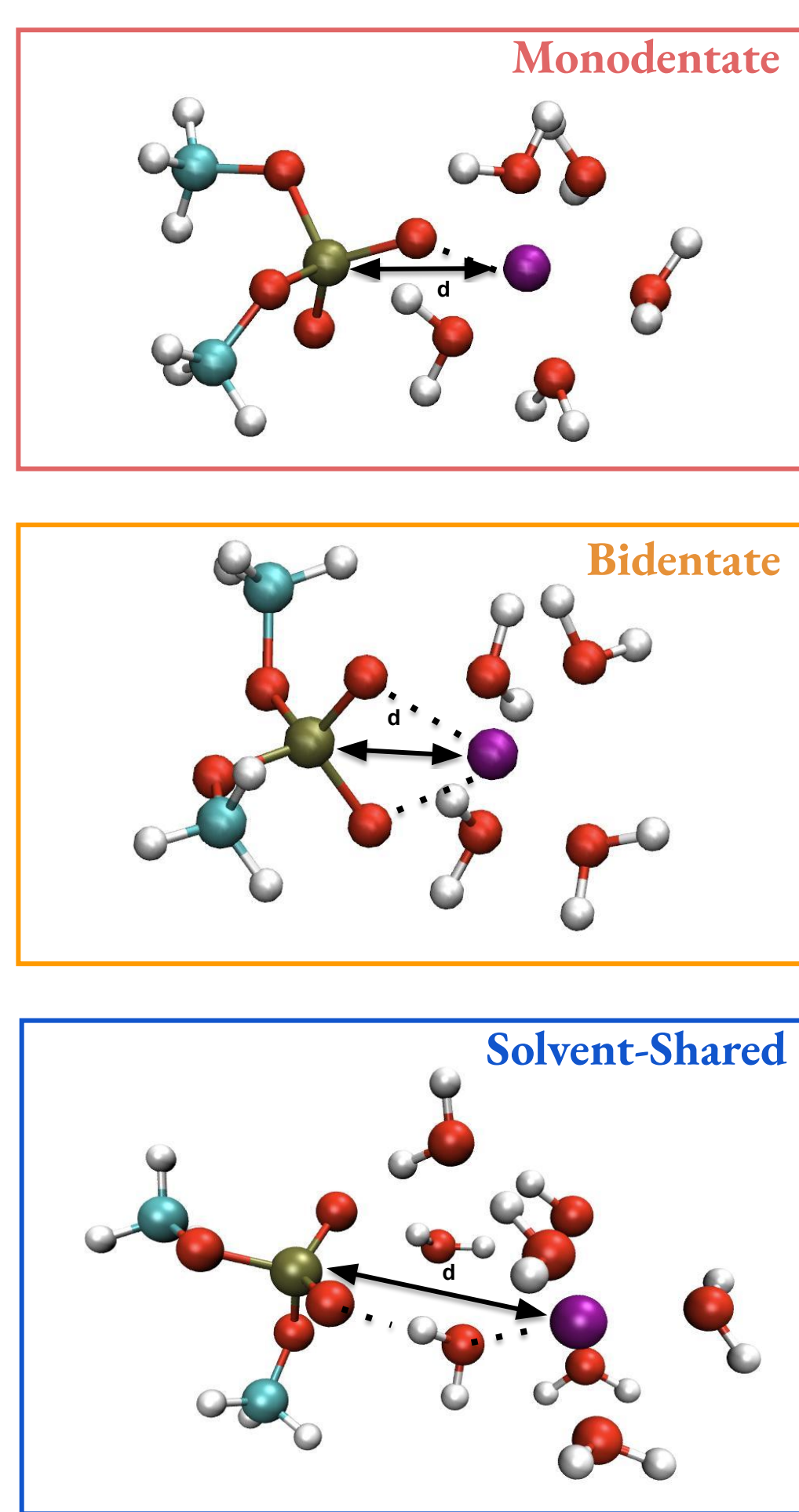
RNA enzymes, called ribozymes, are found to be involved in an increasing number of biological processes. An intriguing question pertains to the **role played by divalent cations** in the ribozyme activity; for the **hammerhead ribozyme**, the cleavage reaction is 500 times faster in the presence of Mg²⁺ than in the presence of Ca²⁺ and 10 000 times faster than in the presence of Na⁺.

We face several challenges: first, the interaction of divalent ions with negatively charged nucleic acids is notoriously poorly described in standard classical simulations due to an oversimplified description of electrostatic interactions, making it difficult to properly capture their slow reorganization along the reaction; here, we thus examine how different strategies to **include electronic polarization effects in force fields**—implicitly through the use of **scaled charges** or **pair-specific Lennard-Jones** parameters, or **explicitly** with polarizable force fields, the **Drude** polarizable force field and **AMOEBA**—capture the interaction of a model phosphate compound, dimethylphosphate. We show that the computational efficiency of implicit models makes them ideally suited for large-scale simulations of biological assemblies, with improved accuracy compared to state-of-the-art fixed-charge force fields. Since RNA is very flexible, it is **necessary to properly sample their conformations** with the use of specific tools (**REST2**) to characterize the **conformational fluctuations** of the **active site** in the presence of different cations. From the main identified conformations, the **Adaptive String Method** with a **QM(DFTB3)/MM MD** description is used in order to study the **reaction mechanism**.

How to describe cations-RNA interactions?

Model system : Dimethyl Phosphate

Binding geometries



Experimental values

Mg²⁺ : $\Delta G^{\circ}_{\text{binding}}$: -7.1 kJ/mol
 Ca²⁺ : $\Delta G^{\circ}_{\text{binding}}$: -5.9 kJ/mol

Goal

Reproduce Theoretically

Verbeek, R. M.; De Bruyne, P. A.; Driessens, F. C.; Verbeek, F. *Inorg. Chem.* 1984, 23, 1922–1926
 Medowell, H.; Brown, W. E.; Sutter, J. R. *Inorg. Chem.* 1971, 10, 1638–1643.

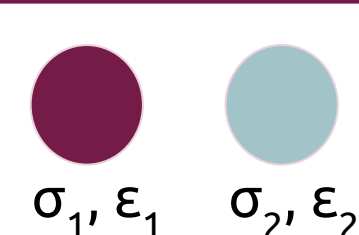
How to describe the electronic polarization?

Standard force field

Lack of electronic polarization

Implicit polarisation

• Specific pair Van der Waals interactions



$\sigma_{12} = \frac{1}{2}(\sigma_1 + \sigma_2)$
 $\epsilon_{12} = (\epsilon_1 \epsilon_2)^{1/2}$ → $\sigma_{12}, \epsilon_{12}$: Specific Values

• Scaled charges : "Electronic Continuum Correction"

$\epsilon_{\infty} = 1.8$

Charge scaled by $1/\sqrt{\epsilon_{\infty}} \approx 0.75$ $|F_{ECC}| = \frac{1}{4\pi\epsilon_0} \frac{q_1}{r^2} \frac{q_2}{r^2}$

Fyta, Netta, *J. Chem. Phys.*, 2011, 136, 124103

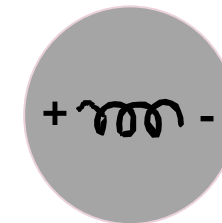
Leontyev, Stuchebnikov, *Phys. Chem. Chem. Phys.*, 2011, 13, 2613–2626

Yoo, Akimsteinov, *Phys. Chem. Chem. Phys.*, 2018, 20, 8432

Polarizable force field

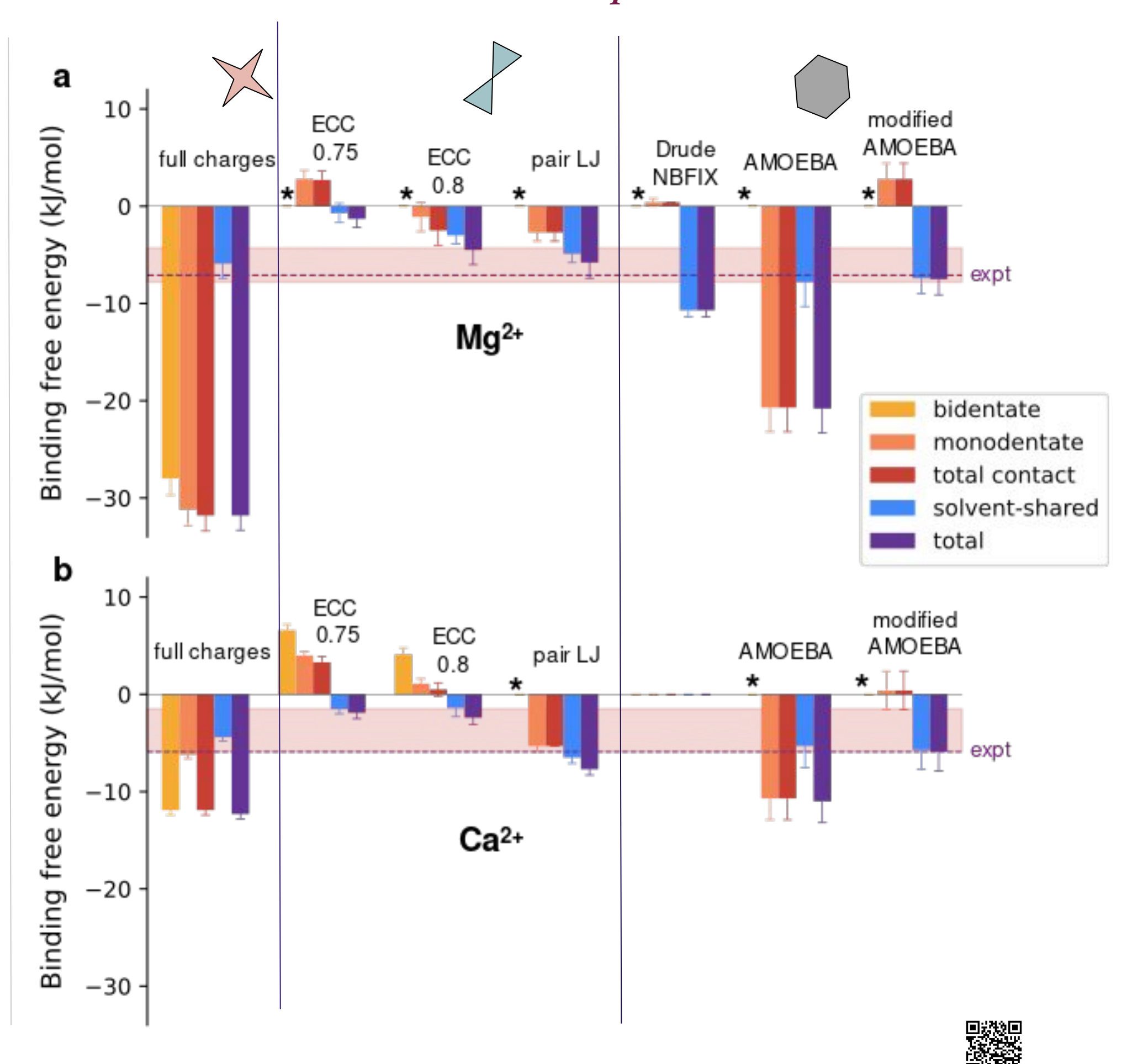
• AMOEBA (e.g induced dipoles)

• Drude Model



Ponder, ..., T. Head-Gordon, *J. Phys. Chem. B*, 2010, 114, 2549–2564
 Lemkul, Huang, Roux, MacKerell, *Chem. Rev.*, 2016, 13, 2613–2626

Comparison of the different approaches
 Alchemical transformations
 in each ion pair

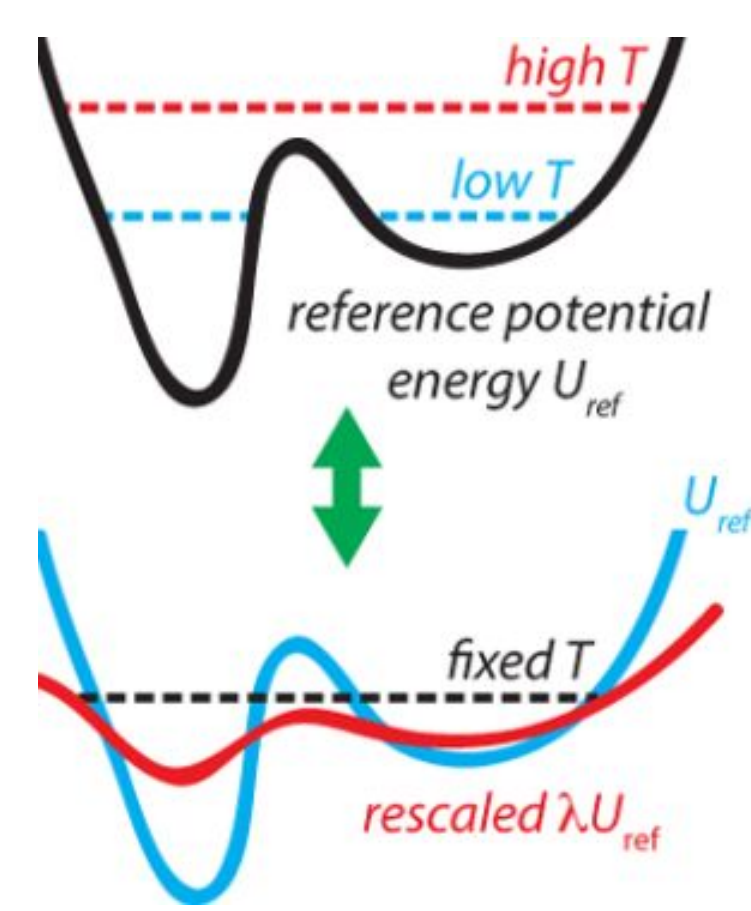


How to sample the conformational landscape?

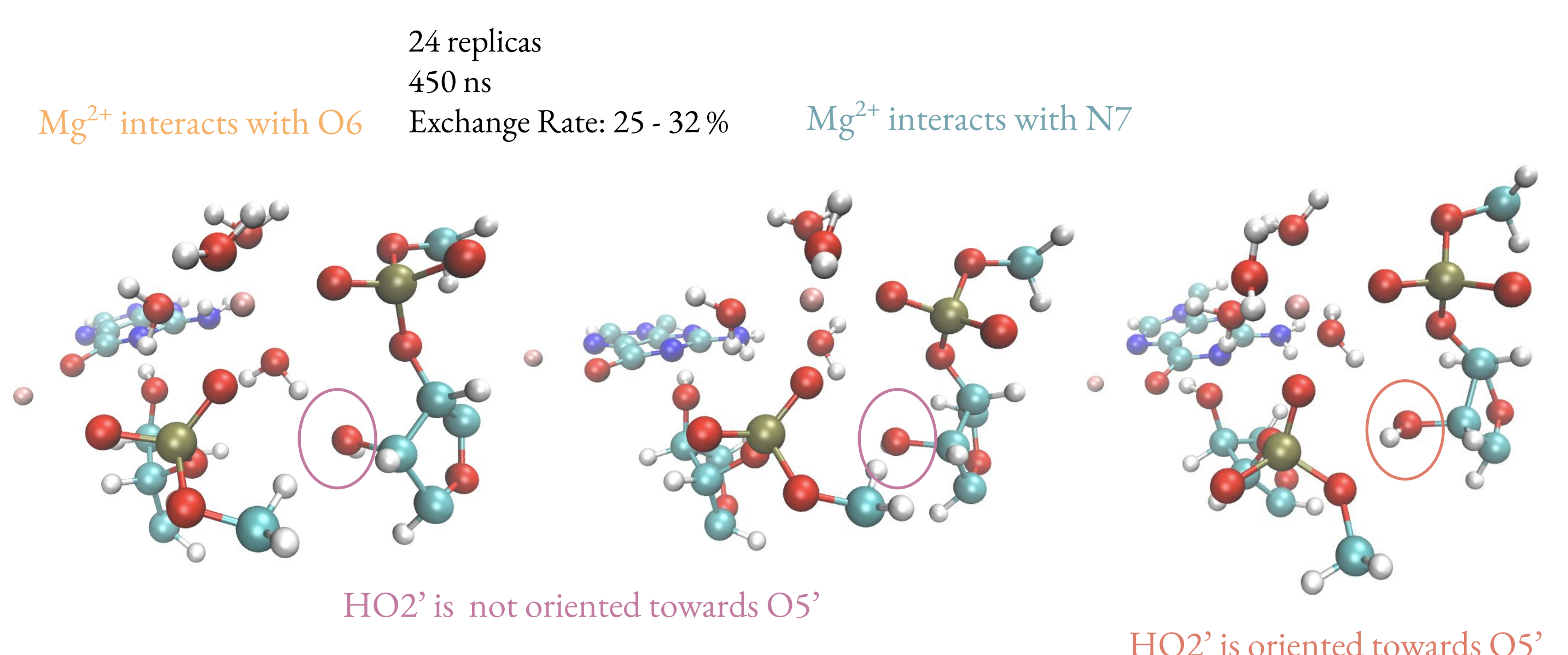
Replica Exchange with Solute Tempering 2

$$E_i(\vec{X}) = \lambda_i E_{pp}(\vec{X}) + \sqrt{\lambda} E_{pw}(\vec{X}) + E_{ww}(\vec{X})$$

$E_{pp}(\vec{X})$, $E_{pw}(\vec{X})$, and $E_{ww}(\vec{X})$ respectively designate the protein–protein, protein–water, and water–water potential energies

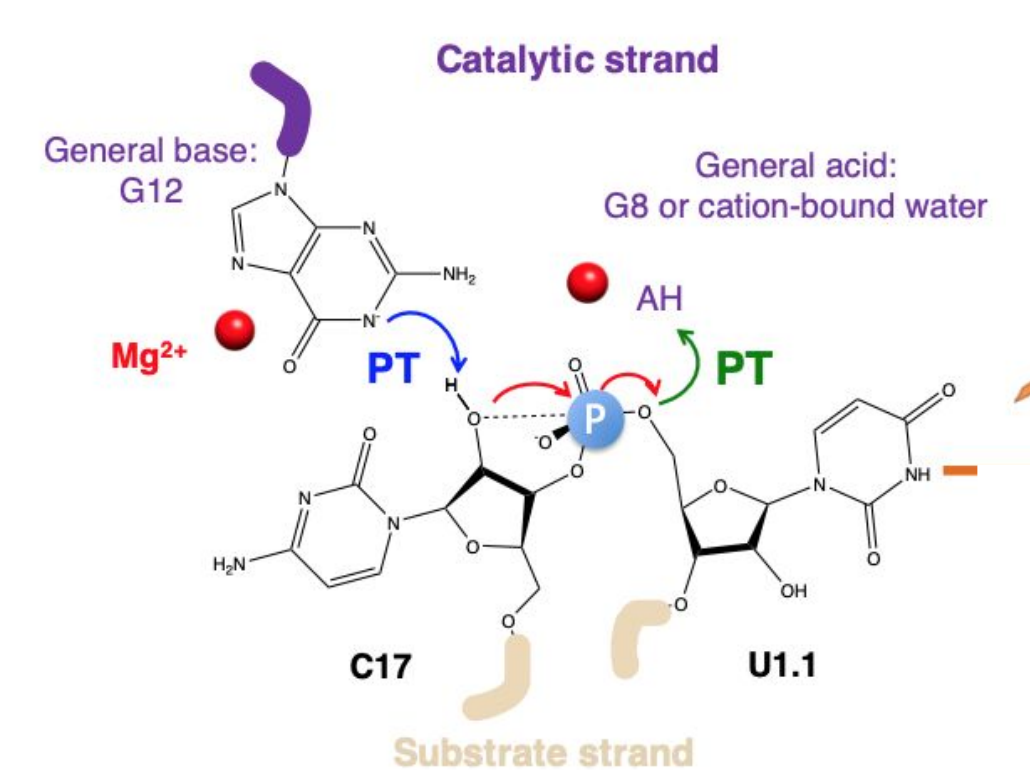


Stirnemann, G; Sterpone, F, *J. Chem. Theory Comput.* 2015, 11, 12, 5573–5577



How to Explore the Reaction Mechanism?

Suggested mechanism

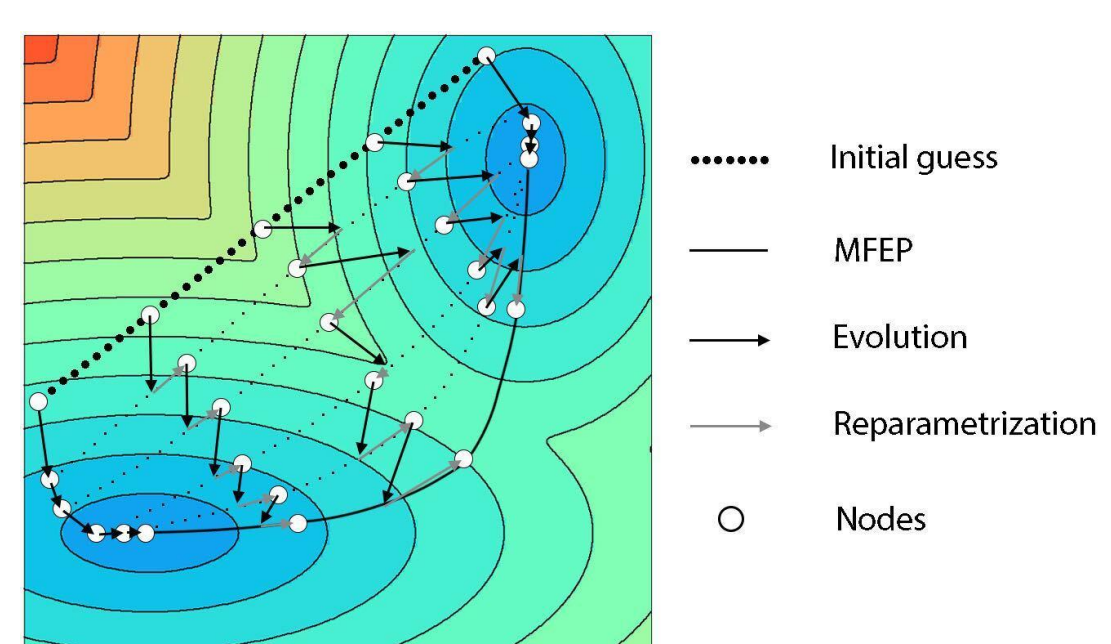


- 2 Proton Transfers and a Nucleophilic Attack
- What is the **Influence of the Ions** on the **Catalytic Activity**? (nature, barriers, concerted or sequential mechanism?)

Roychowdhury-Saha, M.; Burke, D. H. *RNA*, 2006, 12, 1846–1852; Wong, York, *Biochem. J.* 2011, 7, 1; Mir, A.; Chen, J.; Robinson, K.; Lendy, E.; Goodman, J.; Neau, D.; Golden, B. L. *Biochemistry*, 2015, 54, 6369–6381

Adaptative String Method

Dynamic exploration based on QM/MM MD



- Restrained MD Simulations
- **Convergence to the MFEP**
- Umbrella Sampling with Hamiltonian Replica Exchange → sample the Configurational Space

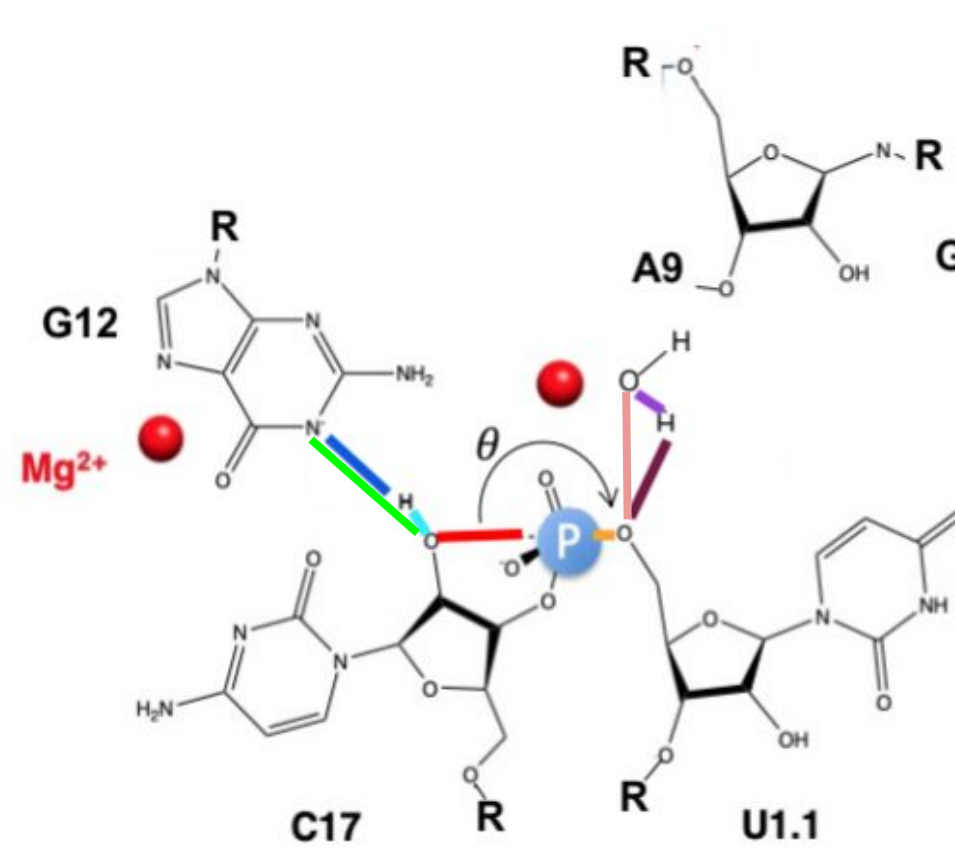
Mir, A.; Golden, B. L. *Biochemistry*, 2016, 55, 633–636; Zinoviev, K.; Tuñón, I. *J. Phys. Chem. A*, 2017, 121, 9764–9772

Preliminary results

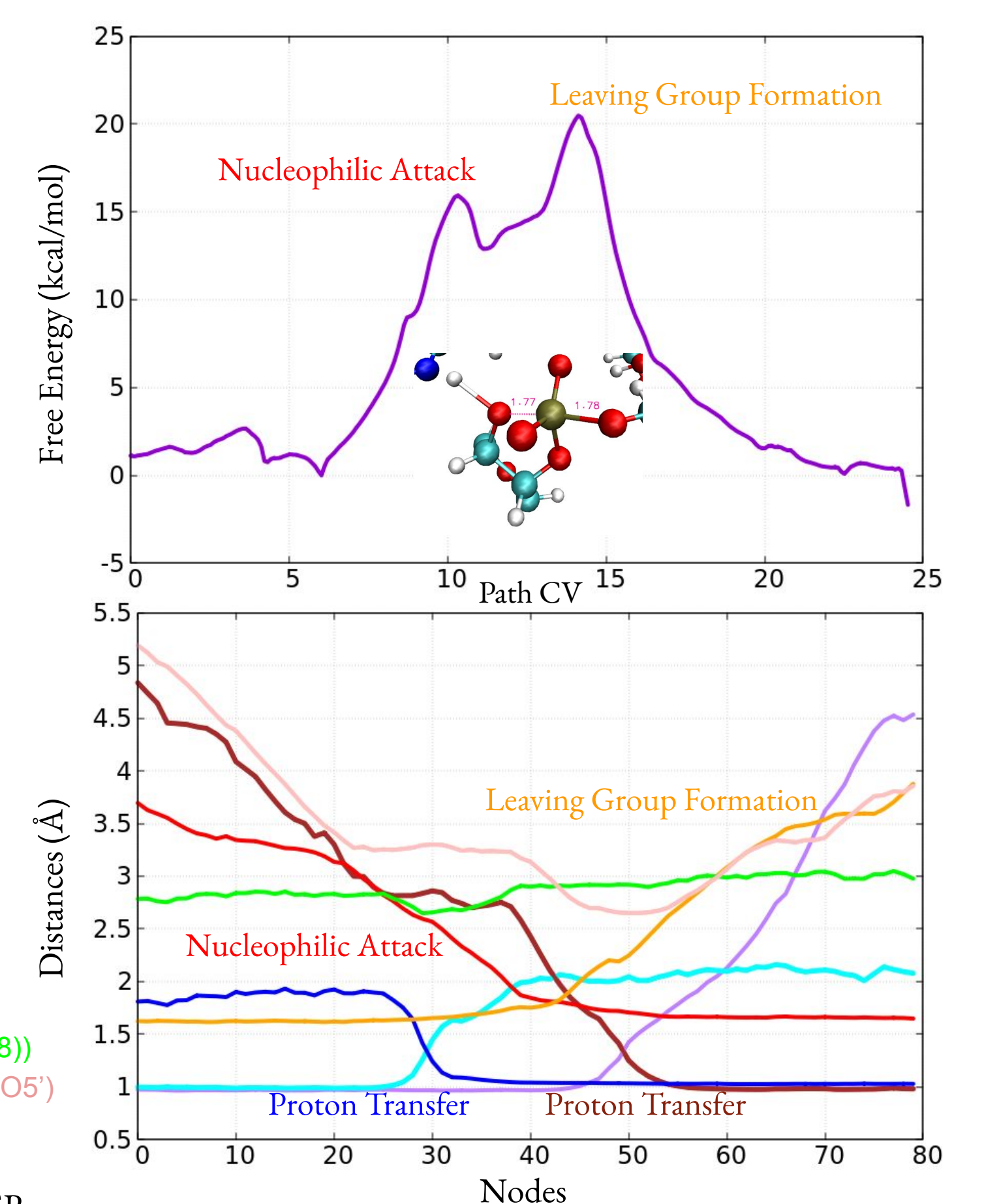
Total : 97 739 atoms
 QM part: 75 atoms
 QM theory: DFTB3
 MM : amberff99-bsc0- $\chi_{OL3}(\epsilon_{OL1}^{\zeta}, \epsilon_{OL1}^{\beta}, \epsilon_{OL1}^{\gamma})$

QM/MM convergence
 Time step: 1 fs
 Duration: 120 ps

Umbrella Sampling
 Time step: 0.5 fs
 Duration: 80 ps



Software: AMBER



Conclusion and Perspectives

- Implicit Description of the Polarization successfully reproduce the Binding Free Energy : more adapted for Large Scale Biochemical Assemblies
- Challenges: lack of Transferability for the Pair-Specific LJ approach, and Scaling of Biomolecular Charges for ECC
- For the tested conformation: concerted mechanism with phosphorane-like intermediate
- Explore mechanistic routes by changing the order of the events, the type of mechanism (Ssequential or concerted), the Nature of the Ions...