

STOCHASTIC BOUNDARY MOLECULAR DYNAMICS SIMULATION OF GDP + Pi + RAB-11 COMPLEX



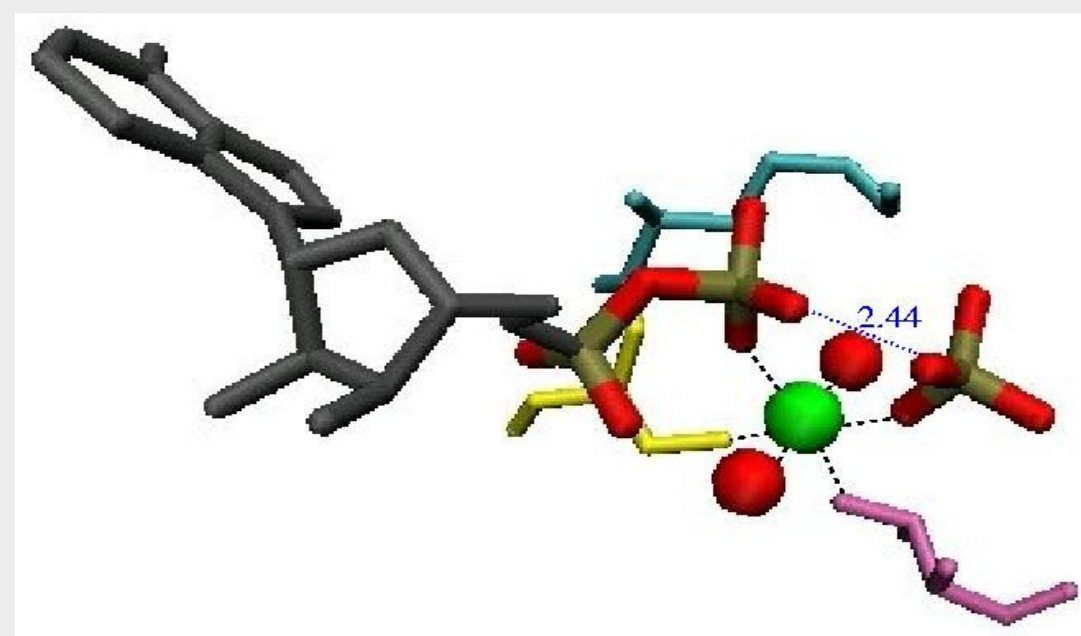
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INTRODUCTION

Rab GTPases, small GTP-binding proteins of the Ras oncogene superfamily cycle between an active state bound to GTP and an inactive state bound to GDP, which form the basis for their ability to function as molecular switches in multiple cellular regulations. The active form is turned off by hydrolysis of GTP. Rab11 GTPase, a member of the Rab protein family, plays a role in regulating various cellular functions, including plasma membrane recycling, phagocytosis, and cytokinesis. Recently, a structure of the complex Rab11-GDP-Pi was obtained⁽¹⁾. It reveals a very short O—O distance (2.44 Å) between Pi and the cleaved oxygen of GDP, a LYS has hydrogen bonds with Pi and GDP, and the distance between the cleaved oxygen of GDP and the phosphate of Pi is 3.3 Å.



Interatomic force due to the interaction between the atoms in the system. This is the same force used in Newton's equation of motion.
Frictional force which describes the drag on the particle due to the solvent. The magnitude of the drag is related to the friction coefficient.
Stochastic force or random due to thermal fluctuations of the solvent. The solvent is not explicitly represented but its effects on the explicit atoms comes from the frictional and random forces.

When the frictional and random forces are zero, the Langevin equation reduces to Newton's equation of motion.

This hybrid method couples the water molecules in the buffer region to a heat bath (reservoir) which keeps the system at thermal equilibrium. A spherical boundary potential of 20 Å is employed to maintain the correct average distribution of water molecules and to prevent water from escaping into the vacuum. The stochastic boundary approach removes water molecules that are far from the area of interest, reducing the number of explicit water molecules in the simulation while still including, in an approximate way, their effect on the molecules in the reaction region.

We have run dynamics of 110 ps to equilibrate the system and 600 ps to make the production, with the *charmm*^(2,3,4) program. The temperature of the Langevin thermal bath is 300K. A cutoff of 12.0 Å is applied to the non-bonded interactions and for the interactions outside this range the extended electrostatics method is used.

METHODS

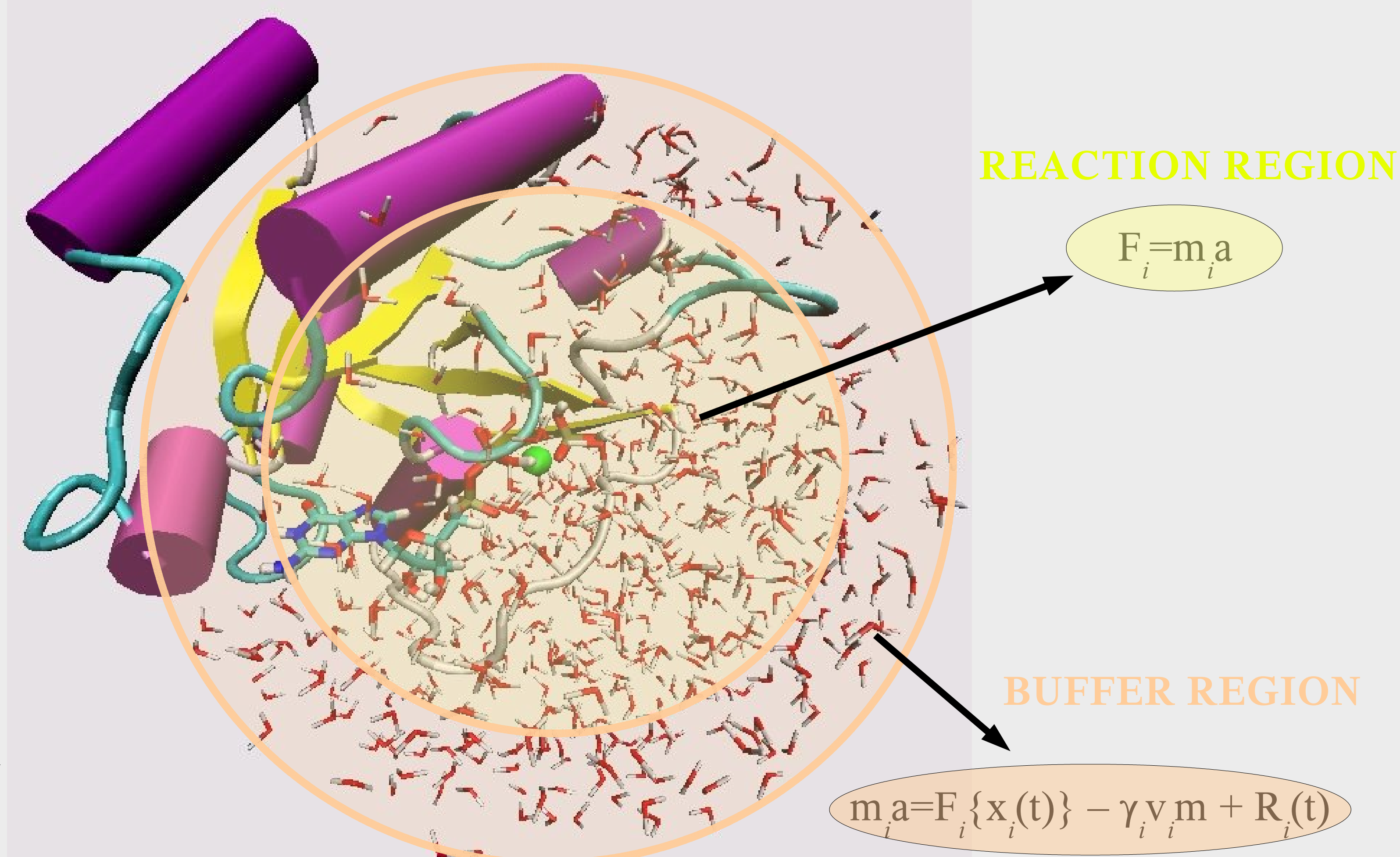
STOCHASTIC BOUNDARY MOLECULAR DYNAMICS:

We have solvated a part of the protein with a 20 Å sphere of water around the Mg²⁺ ion. The protein is divided into 3 regions, an inner **reaction region** of 16 Å, an outer **buffer region** of 20 Å and the outer region, the **reservoir**. In the reaction region, the molecular dynamics simulations are done in the conventional way, using Newton's equations of motion.

$$F_i = m_i a_i$$

In the buffer region, the molecular dynamics are treated explicitly using the Langevin equations of motion:

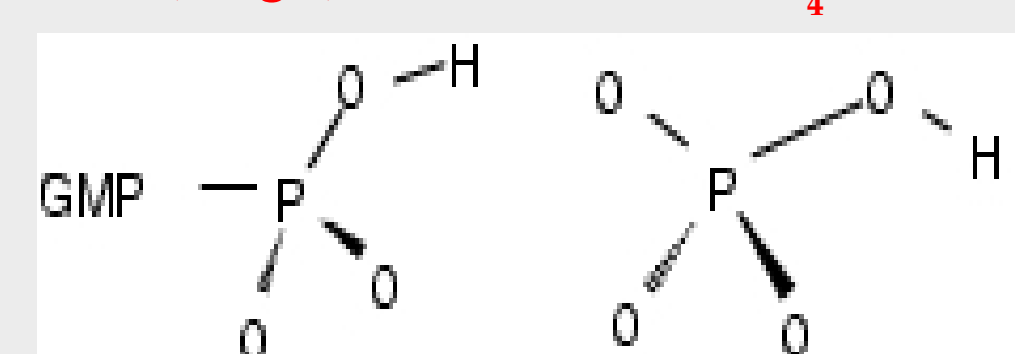
$$m_i a_i = F_i \{x_i(t)\} - \gamma \dot{v}_i m_i + R_i(t)$$



RESULTS

We considered different protonation states of GDP and phosphate, and perform SBMD simulations to gain insight into the possible protonation state of the Rab11-GDP-Pi structures. This can give us important information to interpret and distinguish among the various possible mechanism of the hydrolytic reaction.

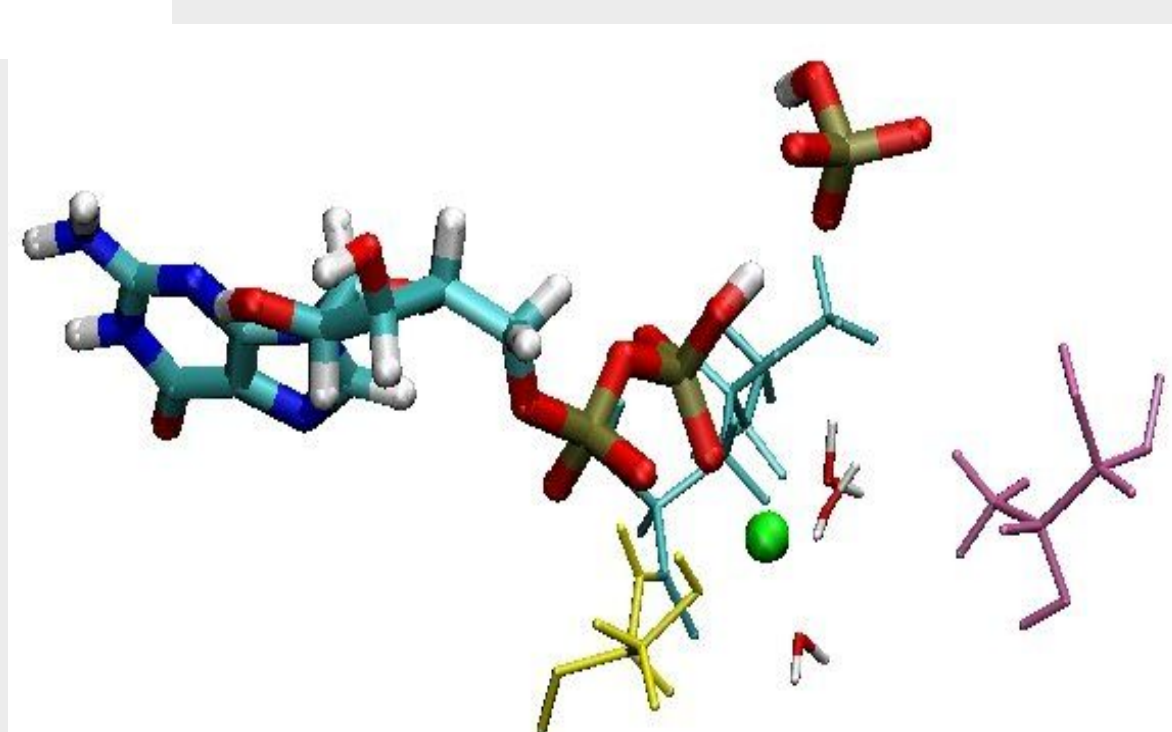
1- 1oix⁻¹, Mg⁺², GDPH⁻² and HPO₄⁻². Total charge -3.



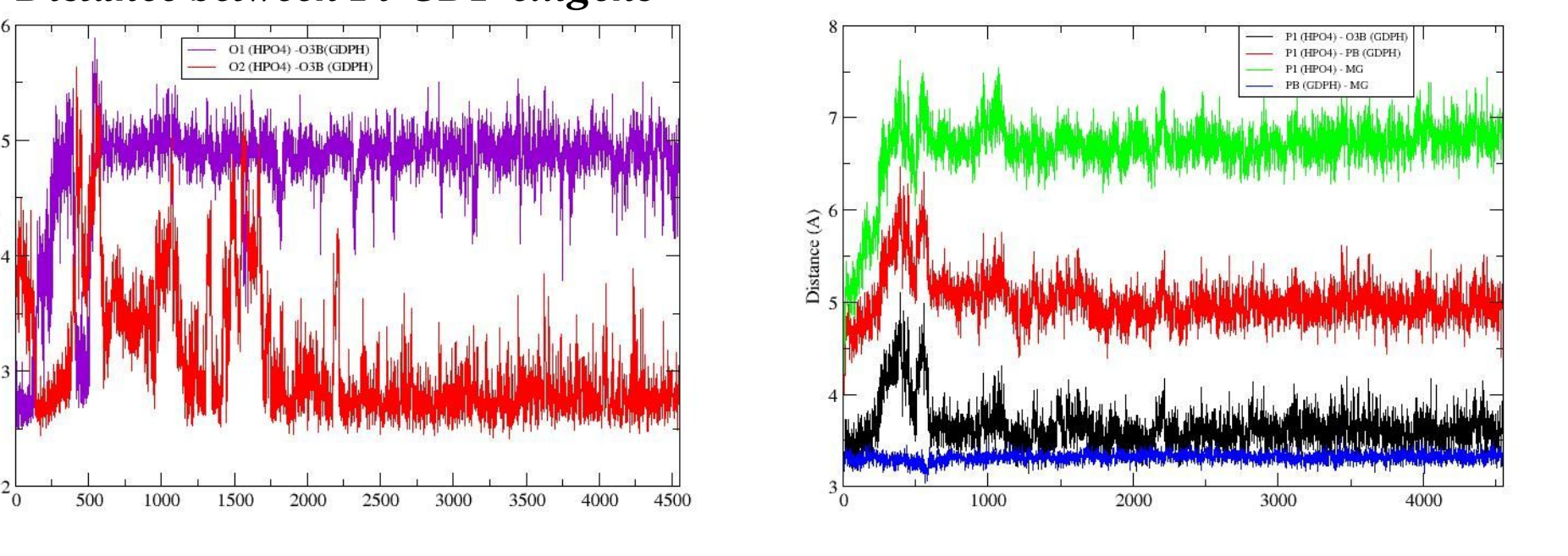
HPO₄⁻² and THR 38 departs from the Mg⁺². The interaction between LYS 19 and GDPH⁻² and HPO₄⁻² is also disrupted and LYS 19 loses its hydrogen bonds with the phosphate moieties.

Average distances

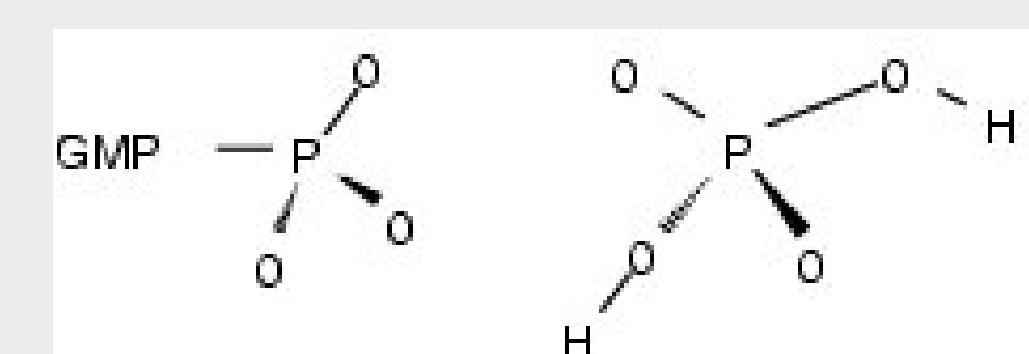
Mg ²⁺	O2B(GDPH ⁻²)	1.790 Å
Mg ²⁺	OG(SER20)	1.986 Å
Mg ²⁺	N(SER20)	2.851 Å
Mg ²⁺	OH2(TIP3 55)	1.948 Å
Mg ²⁺	OH2(TIP3 26)	1.988 Å
Mg ²⁺	OH2(TIP3 87)	1.961 Å
Mg ²⁺	P1(HPO ₄ ⁻²)	6.668 Å
Mg ²⁺	PB(GDPH ⁻²)	3.315 Å
P1(HPO ₄ ⁻²)	O3B(GDPH ⁻²)	3.644 Å
P1(HPO ₄ ⁻²)	PB(GDPH ⁻²)	5.071 Å



Distance between Pi-GDP oxygens



4- 1oix⁻¹, Mg⁺², GDP³ and H₂PO₄⁻¹. Total charge -3

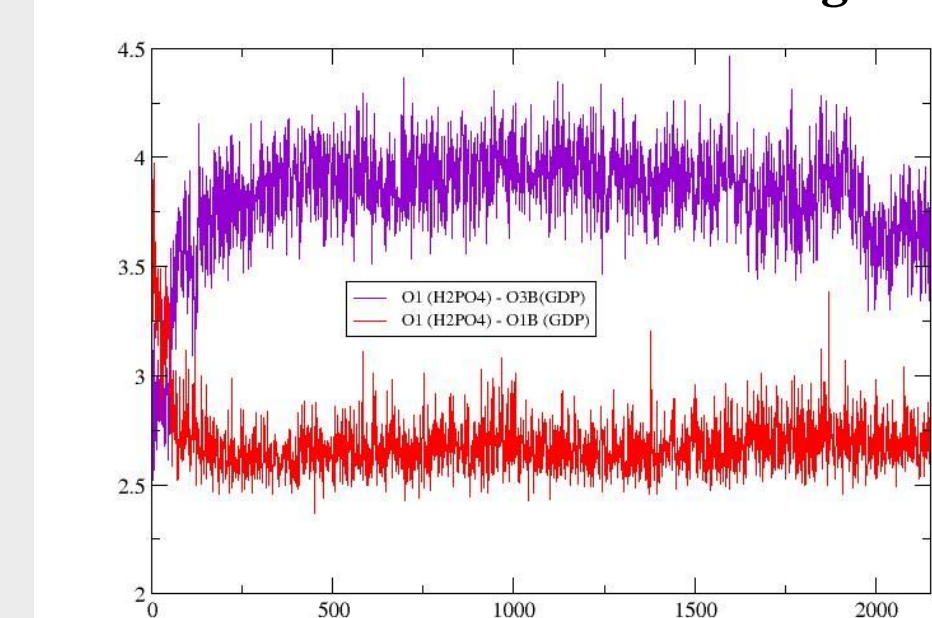


GDP³ bond the Mg⁺² ion and water molecules enter the active site, displacing the SER 20 and THR 38. LYS 19 also departs from GDP³ or H₂PO₄⁻¹. It maintains a short O—O distance and the relative position of both phosphates as in the X-ray structure, although there are also differences (bidentate interaction of GDP³ with Magnesium).

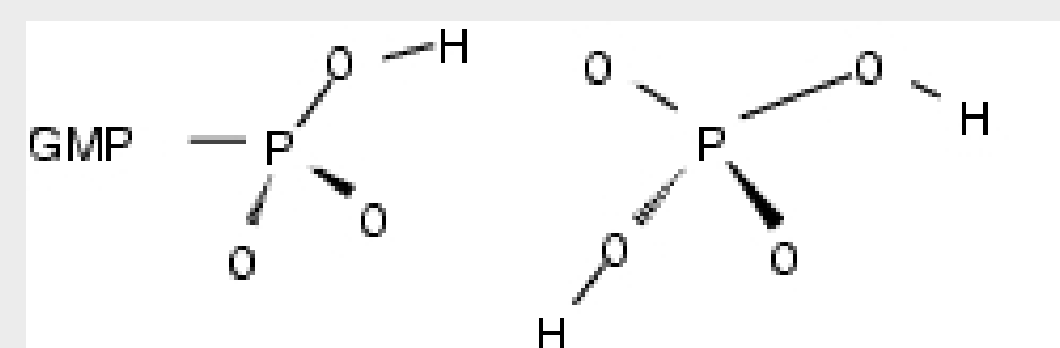
Average distances

Mg ²⁺	O2B(GDP ³)	1.862 Å
Mg ²⁺	O3B(GDP ³)	1.879 Å
Mg ²⁺	O2(H ₂ PO ₄ ⁻¹)	1.852 Å
Mg ²⁺	OH2(TIP3 463)	3.675 Å
Mg ²⁺	OH2(TIP3 85)	1.993 Å
Mg ²⁺	OH2(TIP3 55)	1.970 Å
Mg ²⁺	P1(H ₂ PO ₄ ⁻¹)	3.533 Å
Mg ²⁺	PB(GDP ³)	3.878 Å
P1(H ₂ PO ₄ ⁻¹)	O3B(GDP ³)	3.229 Å
P1(H ₂ PO ₄ ⁻¹)	PB(GDP ³)	2.532 Å

Distance between Pi-GDP oxygens



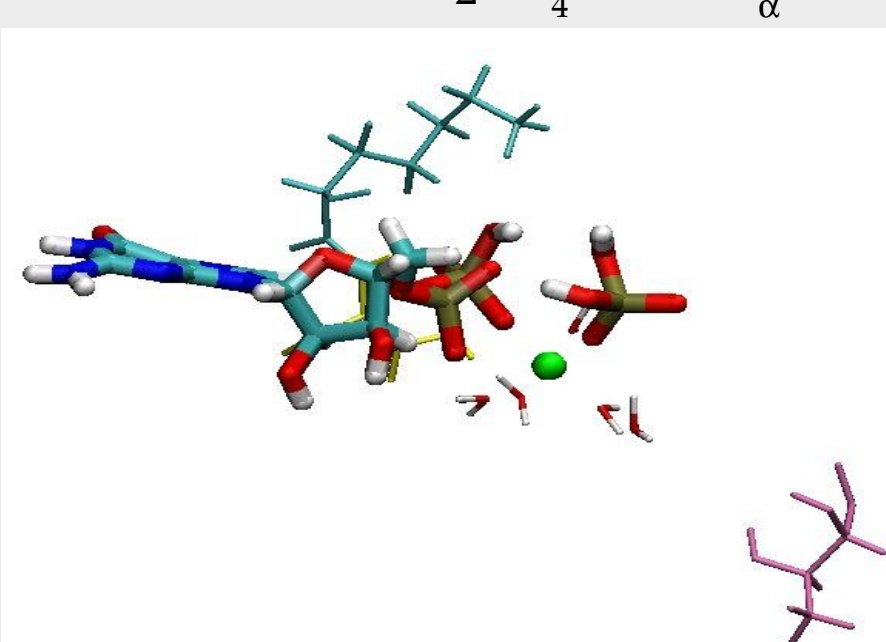
2- 1oix⁻¹, Mg⁺², GDPH⁻² and H₂PO₄⁻¹. Total charge -2.



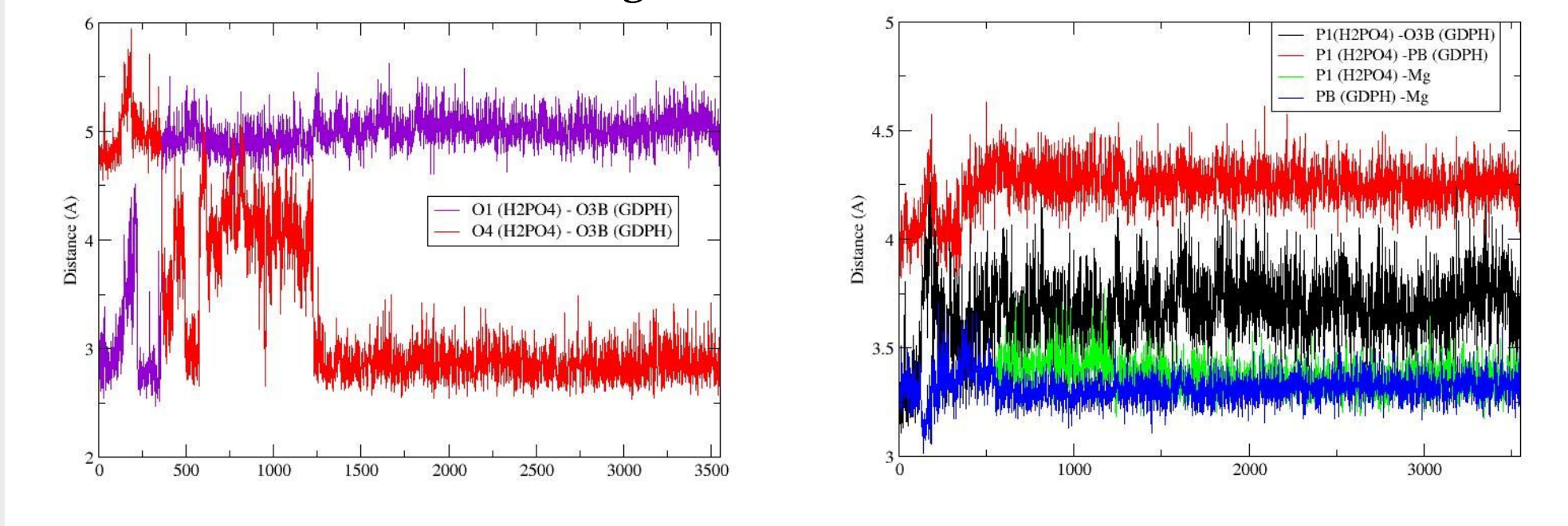
Water molecules enter the active site competing with SER 20 and THR 38 in its interaction with Mg⁺². LYS 19 departs from GDPH⁻² or H₂PO₄⁻¹. Both phosphates, GDPH⁻² and H₂PO₄⁻¹ are maintained in the active site and bound to the Mg⁺². There is also an important interaction between H₂PO₄⁻¹ and P_α of GDPH⁻².

Average distances

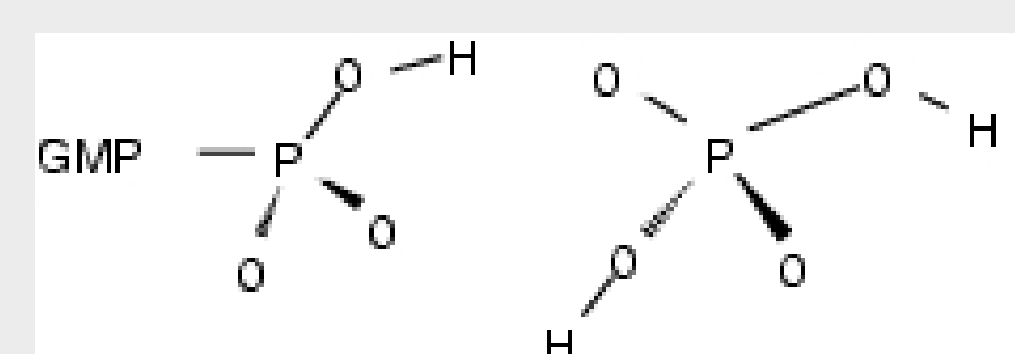
Mg ²⁺	O2B(GDPH ⁻²)	1.825 Å
Mg ²⁺	O2(H ₂ PO ₄ ⁻¹)	1.912 Å
Mg ²⁺	OH2(TIP3 55)	2.011 Å
Mg ²⁺	OH2(TIP3 26)	2.141 Å
Mg ²⁺	OH2(TIP3 38)	2.085 Å
Mg ²⁺	OH2(TIP3 90)	2.001 Å
Mg ²⁺	P1(H ₂ PO ₄ ⁻¹)	3.676 Å
Mg ²⁺	PB(GDPH ⁻²)	4.242 Å
P1(H ₂ PO ₄ ⁻¹)	O3B(GDPH ⁻²)	3.363 Å
P1(H ₂ PO ₄ ⁻¹)	PB(GDPH ⁻²)	3.315 Å



Distance between Pi-GDP oxygens



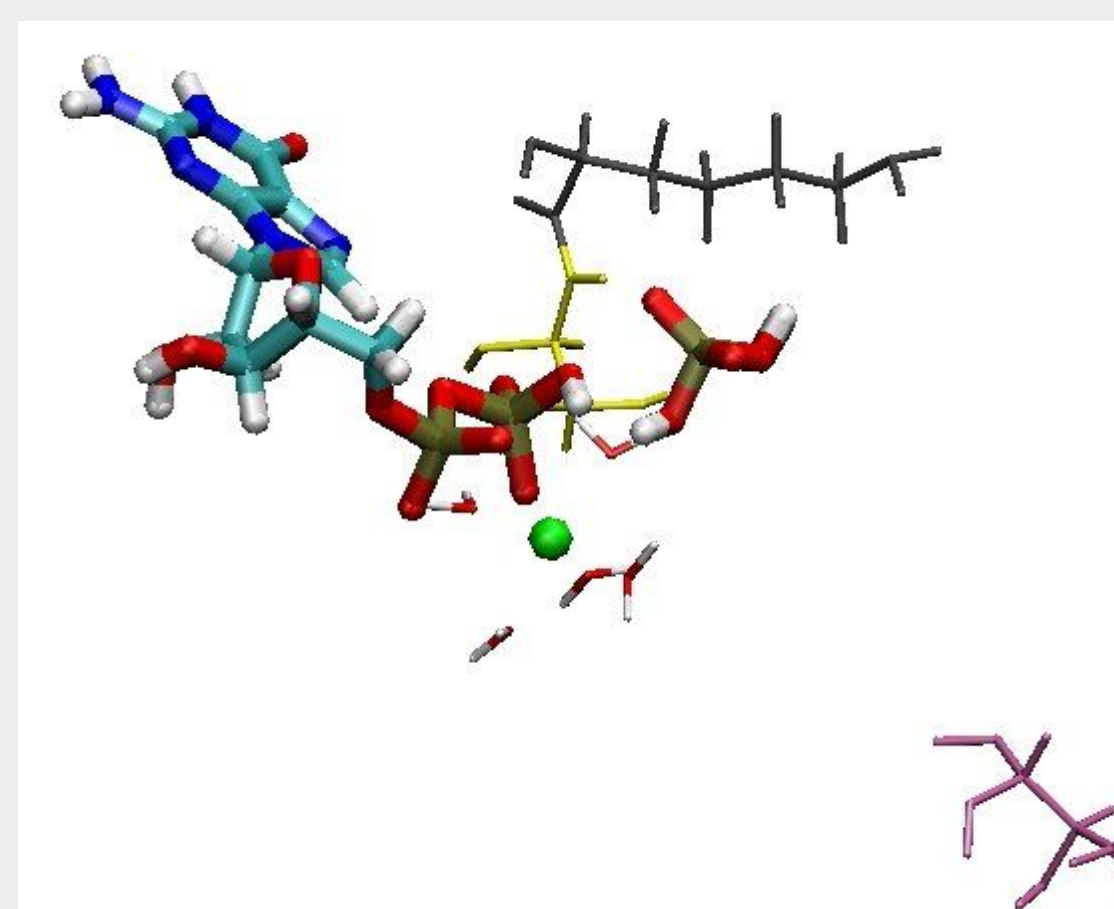
3- 1oix⁻², Mg⁺², GDPH⁻² and H₂PO₄⁻¹. Total charge -3.



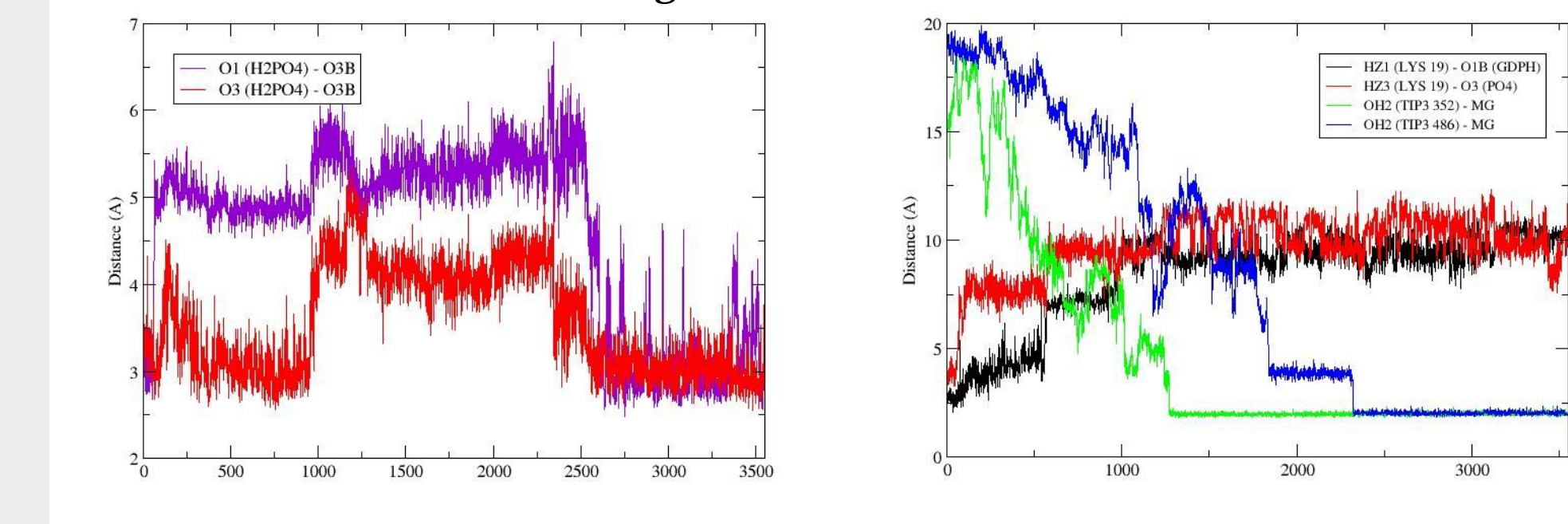
In this case, LYS 19 is neutral. Mg⁺² changes its coordination shell in the production run and coordinates to five water molecules and to GDPH⁻². H₂PO₄⁻¹ lost its direct interactions with Mg⁺². LYS 19 also departs from GDPH⁻² and H₂PO₄⁻¹.

Average Distances

Mg ²⁺	O2B(GDPH ⁻²)	1.803 Å
Mg ²⁺	OH2(TIP3 55)	2.115 Å
Mg ²⁺	OH2(TIP3 88)	2.001 Å
Mg ²⁺	OH2(TIP3 87)	1.967 Å
Mg ²⁺	OH2(TIP3 352)	2.038 Å
Mg ²⁺	OH2(TIP3 486)	1.919 Å
Mg ²⁺	P1(H ₂ PO ₄ ⁻¹)	3.676 Å
Mg ²⁺	PB(GDPH ⁻²)	4.242 Å
P1(H ₂ PO ₄ ⁻¹)	O3B(GDPH ⁻²)	3.363 Å
P1(H ₂ PO ₄ ⁻¹)	PB(GDPH ⁻²)	3.315 Å



Distance between Pi-GDP oxygens



CONCLUSIONS

- Our Stochastic Boundary Molecular Dynamic simulations show that the structure of GDP and Pi in the active site is highly dependant on the protonation state of both phosphate moieties.
 - For the **GDPH⁻² and HPO₄⁻²** state, there is a departure of the HPO₄⁻² phosphate from the active site.
 - For the **GDPH⁻² and H₂PO₄⁻¹** state, both phosphates are stabilized in the active site, although they show a conformation with differences with respect to the X-ray structure.
 - For the **GDP³ and H₂PO₄⁻¹** state, both phosphates remains bound to the Magnesium in a conformation that resembles the X-Ray structure, except for the bidentate nature of the GFP interaction with Magnesium.

- In all of our structures, some water molecules enter into the active site and disrupt the interaction of the Mg⁺² ion with SER 20 and THR 38.
- In addition, LYS 19 is very flexible during the dynamics run and loses its hydrogen bond with phosphate moieties.
- Further calculations need to be done, including Periodic Boundary simulations to check the suitability of the present approach.

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