

**Electronic Supplementary Information for:**

**Anthrax Edema Factor: An Ion-Adaptive Mechanism of  
Catalysis with Increased Transition State Conformational  
Flexibility**

Gabriel E. Jara and Leandro Martínez\*

*Institute of Chemistry, University of Campinas*

E-mail: leandro@iqm.unicamp.br

Table S1: FEP estimates of the free energy parameters of the reaction, using higher-level QM methods (all values in kcal mol<sup>-1</sup>).  $\Delta G^\circ$ : Overall reaction free-energy;  $\Delta_f G^\ddagger$ : Activation free-energy of the forward reaction.  $\Delta_b G^\ddagger$ : Activation free-energy of the backward reaction. Estimated errors were computed as the sum of standard errors of the means of the values used in each computation.

System	DFT Functional	$\Delta G^\circ$	$\Delta_f G^\ddagger$	$\Delta_b G^\ddagger$
EF·1Mg	B3LYP	6.1 (3.1)	18.6 (2.4)	12.0 (2.7)
	M06	0.6 (3.4)	19.9 (2.6)	18.8 (3.0)
EF·2Mg	B3LYP	-21.2 (3.3)	12.9 (2.6)	34.8 (2.4)
	M06	-22.5 (3.3)	12.6 (2.3)	35.8 (2.4)
mAC	B3LYP	-9.3 (2.8)	28.6 (2.3)	37.1 (2.4)
	M06	-8.5 (2.8)	29.1 (2.3)	36.7 (2.3)

\*To whom correspondence should be addressed

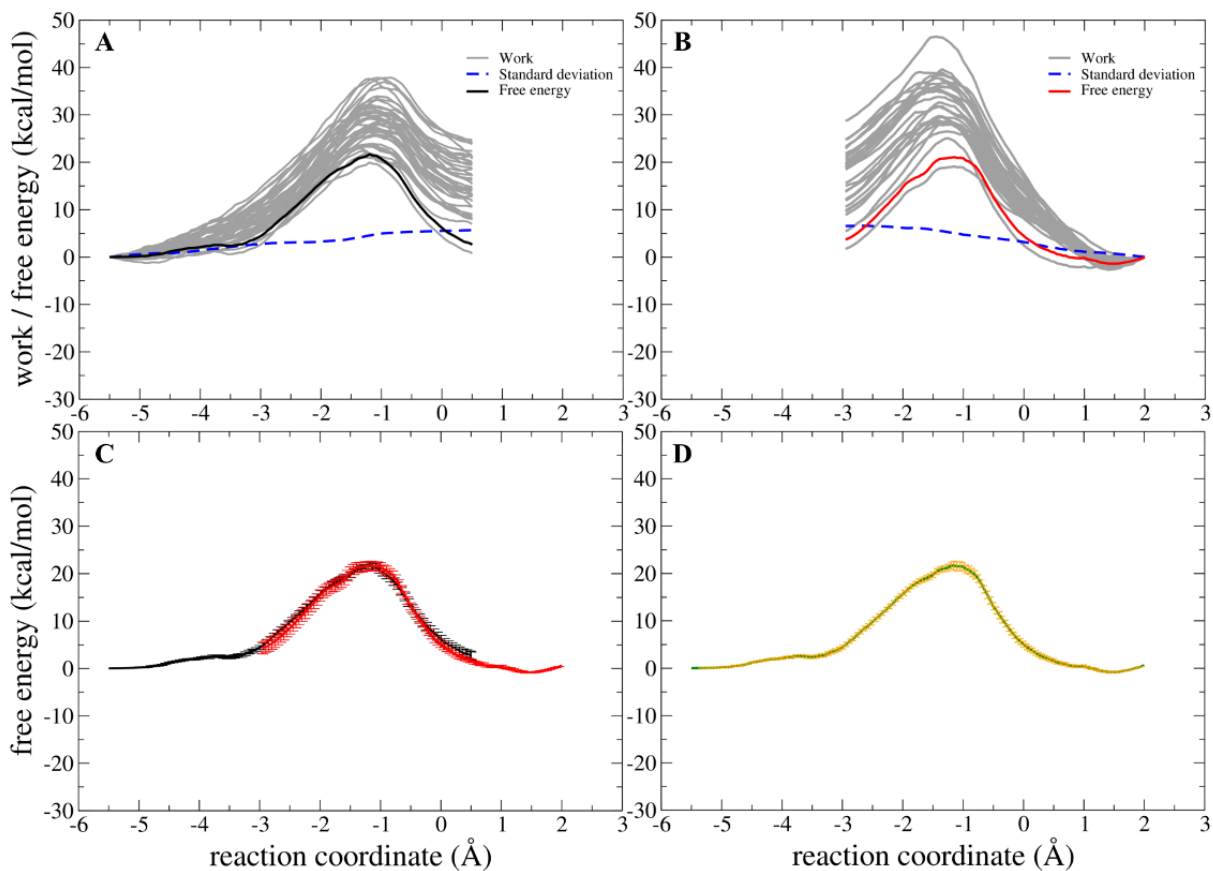


Figure S1: SMD works and free energy profiles for EF·1Mg: A) Forward reaction works for  $\sim 30$  SMD runs (gray), forward reaction free energy profile (black) and standard deviation (blue). B) Same representation for the backward reaction, showing the free energy profile in red. C) Alignment of the forward and backward free energy profiles (black and red) along with the error estimate. D) Final free energy profile. The error estimate is the standard error of the Jarzynski exponential mean, that is,  $\sigma/\sqrt{N}$ , where  $\sigma$  is the standard deviation and  $N$  is the number of independent samples (SMD executions), propagated to first order to the free energy.

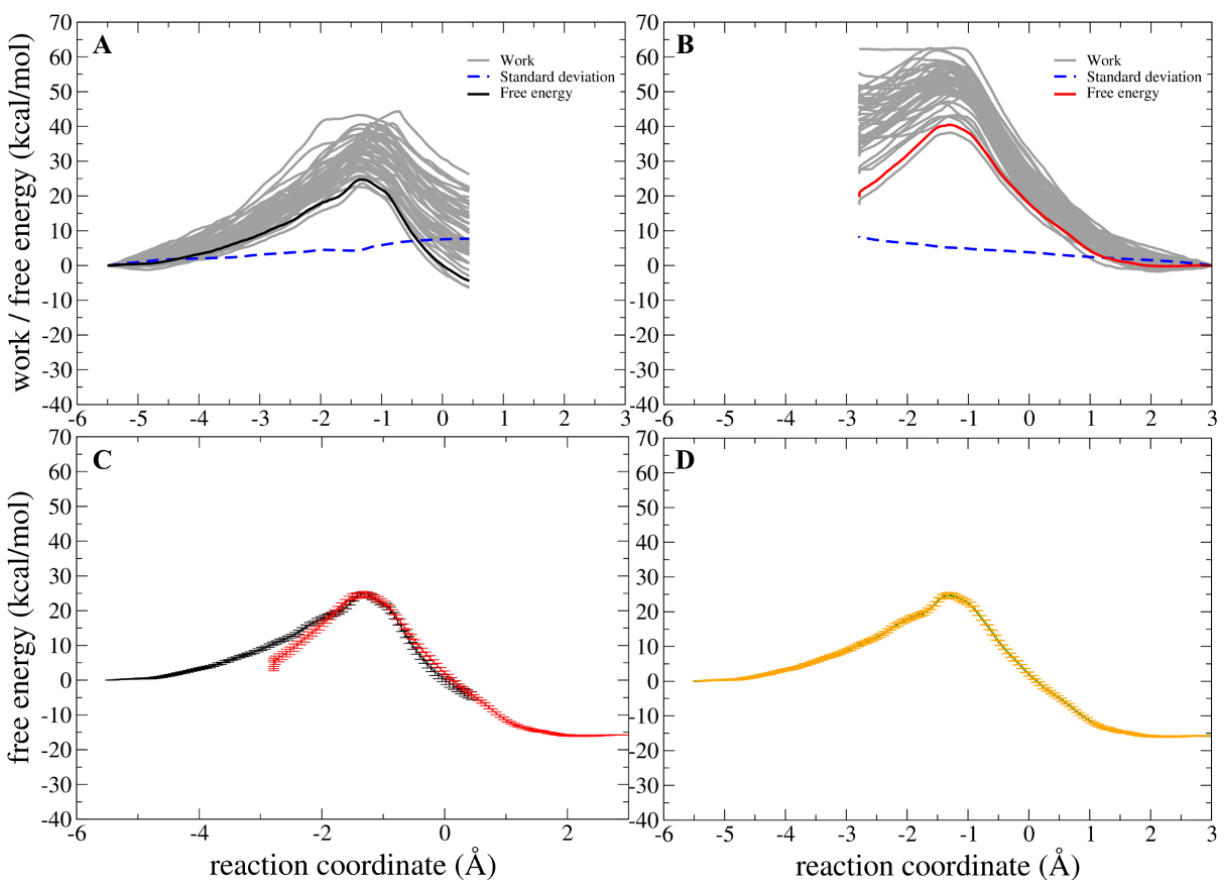


Figure S2: SMD works and free energy profiles for EF·2Mg: A) Forward reaction works for  $\sim 30$  SMD runs (gray), forward reaction free energy profile (black) and standard deviation (blue). B) Same representation for the backward reaction, showing the free energy profile in red. C) alignment of the forward and backward free energy profiles (black and red) along with estimated error bars. D) Final free energy profile.

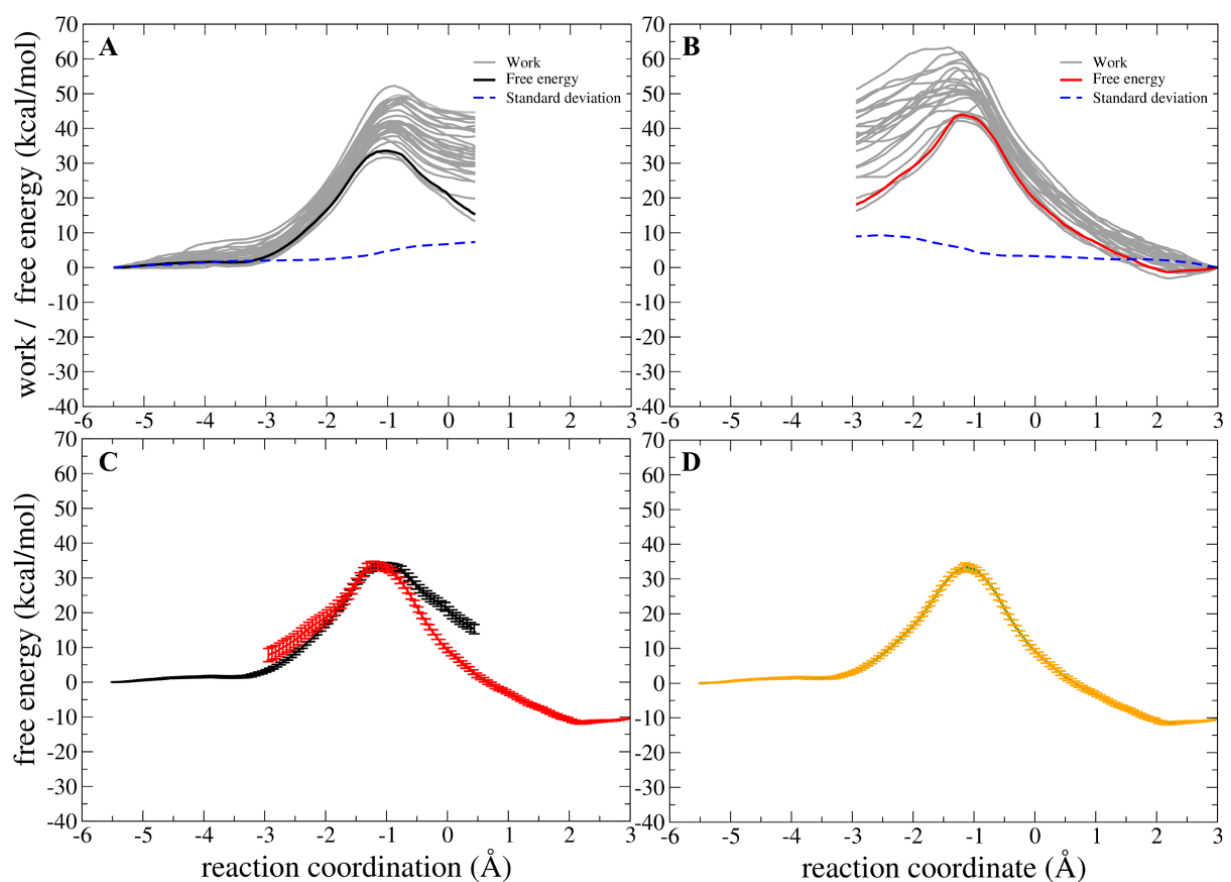


Figure S3: SMD works and free energy profiles for mAC: A) Forward reaction works for  $\sim 30$  SMD runs (gray), forward reaction free energy profile (black) and standard deviation (blue). B) Same representation for the backward reaction, showing the free energy profile in red. C) Alignment of the forward and backward free energy profiles (black and red) along with estimated error bars. D) Final free energy profile.

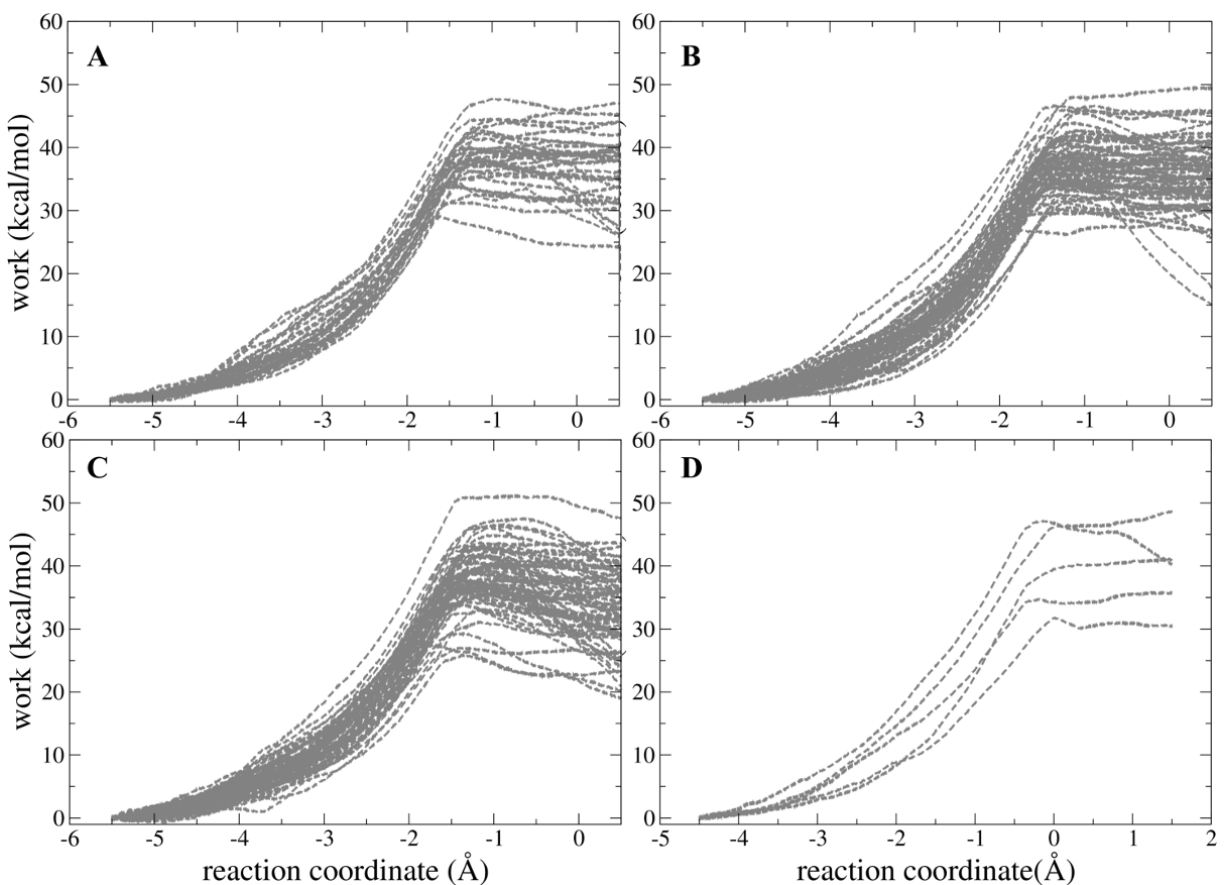


Figure S4: MSMD simulations for the forward reaction of EF·2Mg using a water molecule as base. Essentially no trajectories leading to reaction products could be obtained. For the few productive trajectories obtained, the resulting works were much larger than those obtained for the simulations with His351 acting as the catalytic base. In (A), (B), and (C) plots, we used the same reaction coordinate as the main simulations,  $d(\text{O3}'\text{-P}\alpha) - d(\text{O3}\alpha\text{-P}\alpha) + d(\text{H3}'\text{-Ow})$ . (B) and (C) have different harmonic restrictions over the H3'-Ow distance in order to avoid that the water base diffuses away from the active site. In (D) trajectories were generated using a reaction coordinate including also the O3'-H3' distance.

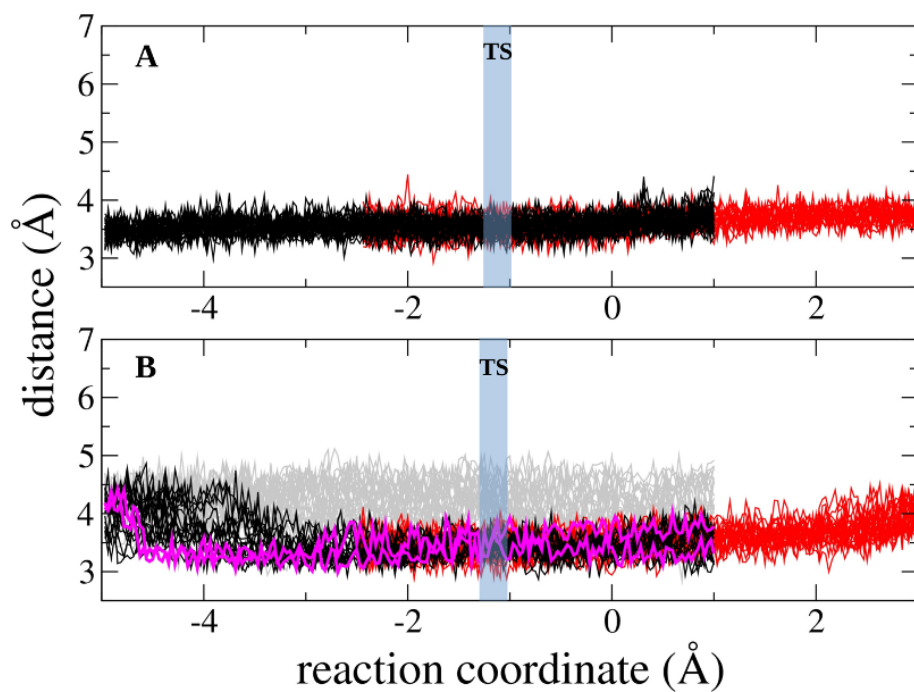


Figure S5:  $\text{Mg}^{2+}$  to  $\text{Mg}^{2+}$  distances in two ion catalytic sites of (A)  $\text{EF}\cdot 2\text{Mg}$  (A) and (B) the mAC. The black and red lines represent the distances measured in the forward and backward reactions, respectively. In the case of  $\text{EF}\cdot 2\text{Mg}$ , the distances observed in the two lowest work trajectories are in magenta, while gray lines represent distances that did not decrease along the forward reaction and are generally associated with larger works. The TS label indicates the transition state region.

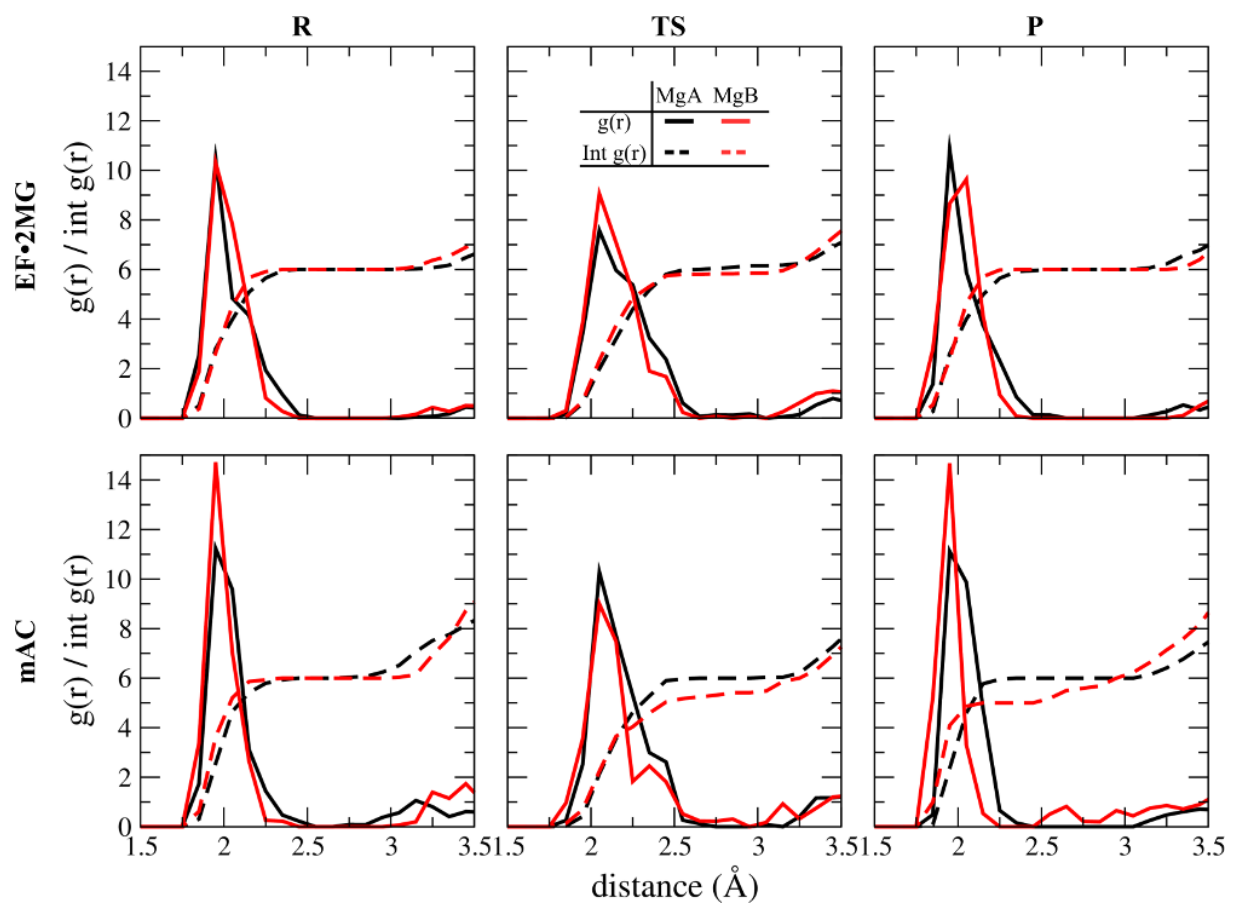


Figure S6: Radial distribution functions ( $g(r)$ ) and their integrals ( $\text{int } g(r) \equiv \int_0^r g(r) dr$ ) for  $\text{Mg}_A$  (black line) and  $\text{Mg}_B$  (red line). The analysis is shown for EF·2Mg and the mAC in the three states: reactants (R), transition state (TS) and products (P).

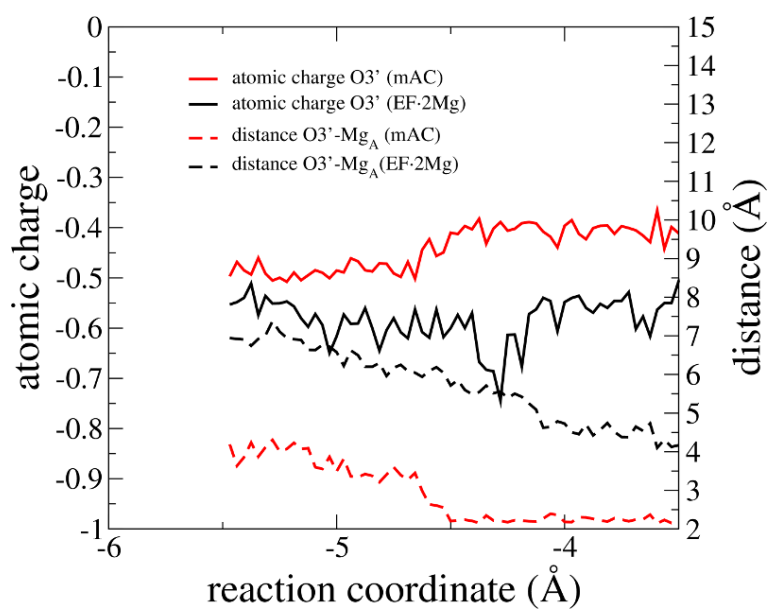


Figure S7: Charges of the O3' atom and O3'-Mg<sub>A</sub> distances for EF·2Mg and the mAC. The figure shows that the coordination of O3' to Mg<sub>A</sub> in mAC involves a negative charge transfer from O3' to Mg<sub>A</sub>. This is not observed in EF·2Mg. The curves are the average results of the three simulations with lowest works.

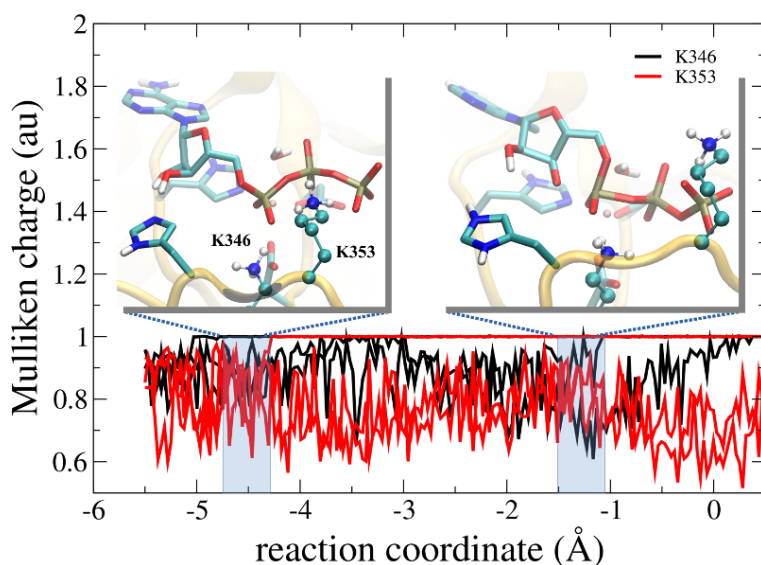


Figure S8: Mulliken charges for K346 and K353 for the EF·1Mg system. The figure shows that the K346 residue assists phosphoryl transfer by accepting negative charge. Besides, the K353 residue stabilizes the pyrophosphate group.



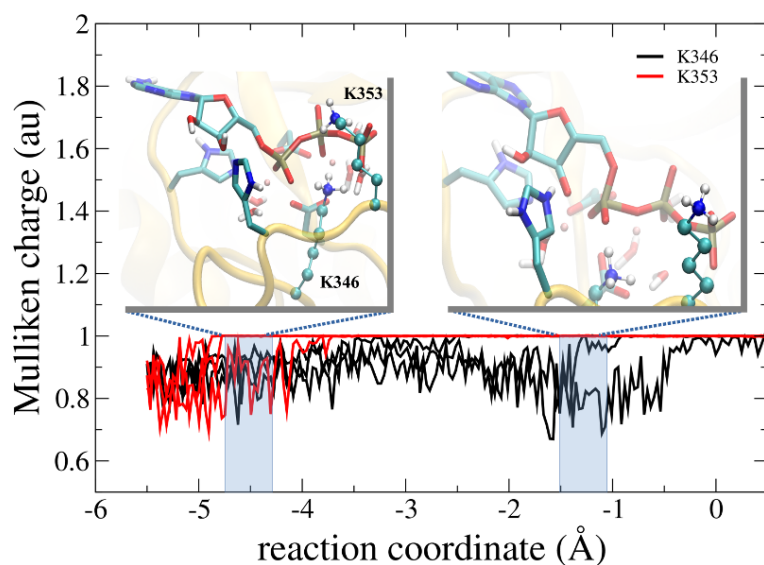


Figure S9: Mulliken charges for K346 and K353 for the EF·2Mg system. The figure shows that the K346 residue assists phosphoryl transfer by accepting negative charge. Besides, the K353 residue stabilizes the pyrophosphate group.

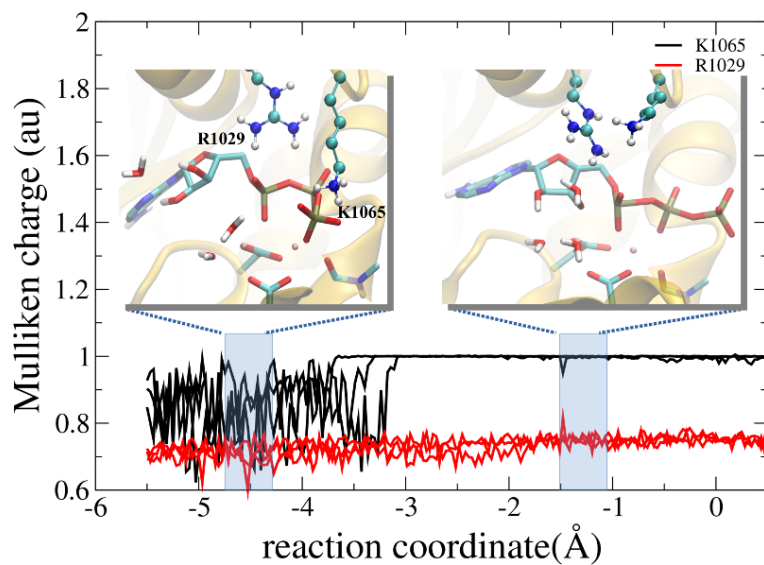


Figure S10: Mulliken charges along the reaction coordinate for R1029 and K1065 for the mAC. The figure shows that the R1029 assists phosphoryl transfer by accepting negative charge. The K1065 residue interacts with the pyrophosphate group only at the beginning of the reaction.