

QM/MM Molecular Dynamics of Proton Transfer in the SARS-CoV-2 3CL Protease

Nicolás Rojo Esteban



1 Introduction

In this work, I employ molecular dynamics (MD) simulations to study the structural changes along the proton-transfer reaction coordinate in the SARS-CoV-2 3CL protease active site.



Figure 1: Reaction mechanism

Specifically, we select three optimized windows corresponding to:

1. **Reactants** (window 1)
2. **Transition State** (window 11)
3. **Products** (window 17)

Each structure was obtained by umbrella-sampling optimization at distinct values of the reaction coordinate and represents the progression from a proton bonded to Cys145 (reactants), through the shared-proton (transition state), to a proton transferred to His41 (products). Then 5 ps QM/MM MD simulations are performed at PM3/MM to compare with the results obtained using AM1/MM.

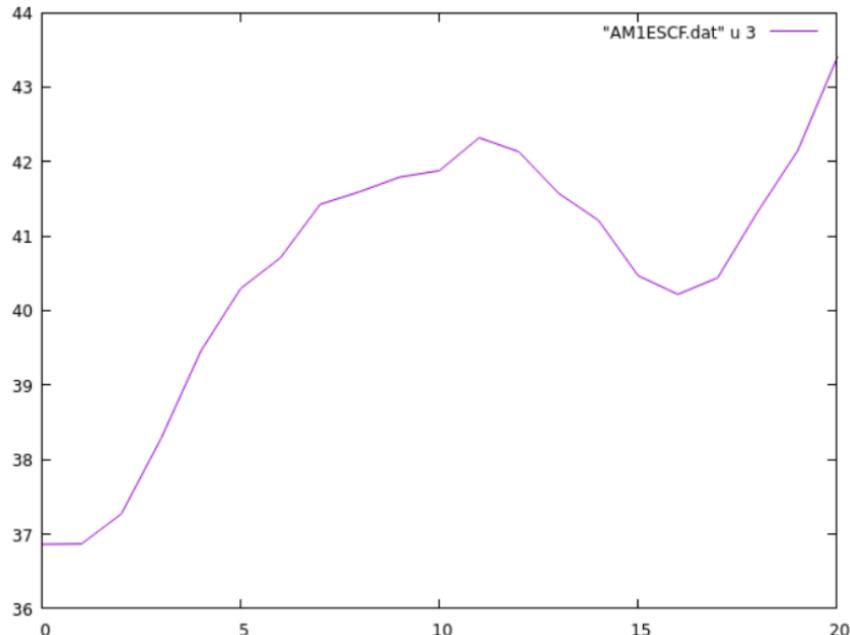


Figure 2: Potential of mean force along the reaction coordinate, showing the 21 umbrella-sampling windows.

2 Results

2.1 PM3/MM calculations

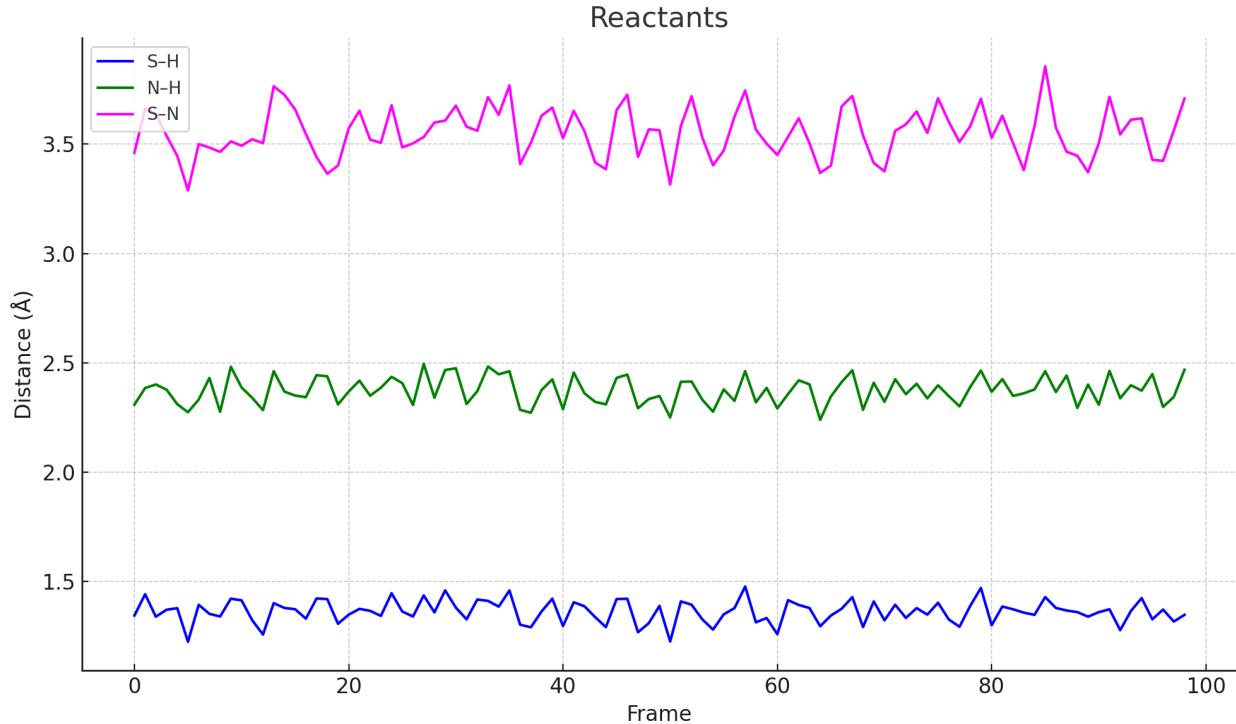


Figure 3: Time evolution of the S–H, N–H and S–N distances in the reactants PM3/MM MD

In the reactant simulation (window 1, $RC \approx -1.0 \text{ \AA}$), the S–H distance remains close to 1.35 \AA , showing that the proton stays on Cys145. The N–H distance is about 2.35 \AA , indicating no proton transfer to His41. The S–N distance is roughly 3.50 \AA , consistent with a disengaged catalytic dyad (Fig. 3). Together, these values confirm that in the reactant state the proton is localized on sulfur and the nitrogen acceptor is not involved.

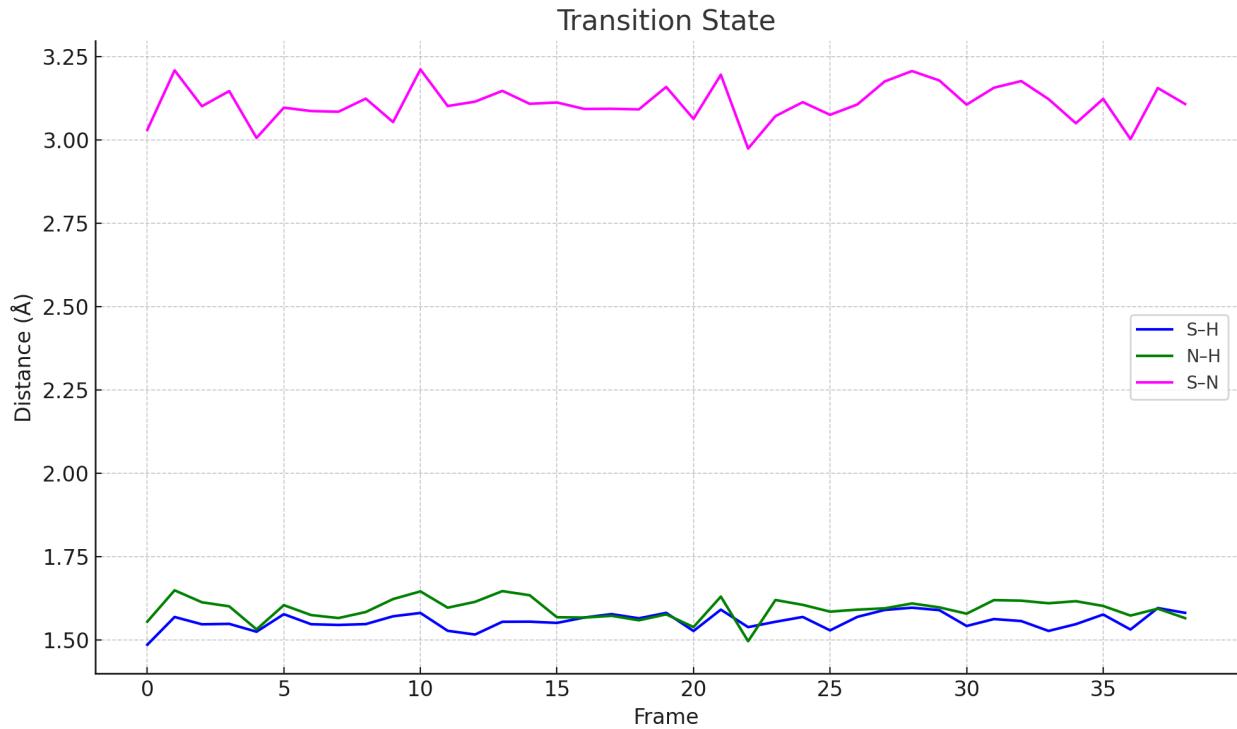


Figure 4: Time evolution of the S–H, N–H and S–N distances in the TS PM3/MM MD

In the transition state simulation, the S–H and N–H distances converge near $1.55\text{--}1.60\text{ \AA}$, indicating that the proton spends time halfway between Cys145 and His41. The S–H distance fluctuates around $1.55 \pm 0.05\text{ \AA}$ while the N–H distance averages $1.60 \pm 0.05\text{ \AA}$. At the same time, the S–N separation remains at approximately $3.10 \pm 0.10\text{ \AA}$ (Fig. 4), shorter than in the reactant state. These values reflect the shared-proton character of the transition state, with neither bond fully formed or broken.

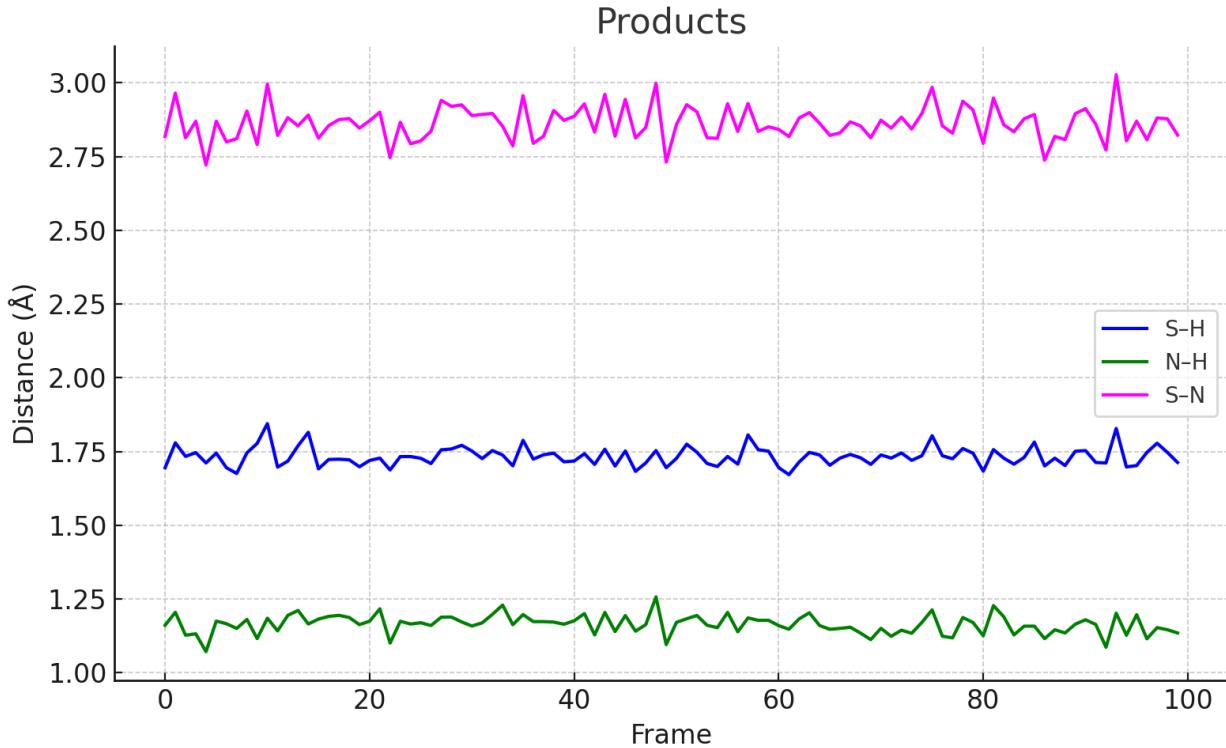


Figure 5: Time evolution of the S–H, N–H and S–N distances in the products PM3/MM MD

In the product simulation, the S–H distance increases to $1.75 \pm 0.05 \text{ \AA}$, indicating cleavage of the S–H bond, while the N–H distance decreases to $1.15 \pm 0.05 \text{ \AA}$, consistent with protonation of His41. The S–N distance averages $2.85 \pm 0.07 \text{ \AA}$, showing that the catalytic dyad has closed around the transferred proton (Fig. 5). These values confirm that in the product the proton resides on the nitrogen atom and the ion pair is fully formed.

2.2 Comparison between PM3/MM and AM1/MM results

PM3 and AM1 are both semi-empirical quantum-chemical methods based on the NDDO (Neglect of Diatomic Differential Overlap) approximation, but they differ in how their core–repulsion and resonance integrals are parametrized. AM1 (Austin Model 1) introduces Gaussian functions to correct the core repulsion between atom pairs, improving predictions of heats of formation over its predecessor MNDO[1]. PM3 (Parametric Method 3) retains the same functional form as AM1 but reoptimizes all core–repulsion parameters against a larger and more diverse experimental dataset, often yielding better geometries and thermochemical properties for organic molecules[2]. In practice, PM3 tends to give slightly more accurate bond lengths and reaction energies, while AM1 remains faster.

These three figures show the variation of distance over time for S–H, N–H and S–N in the reactant, transition state and product simulations using both AM1/MM (dashed lines) and PM3/MM (solid lines). In all three states, the two semi-empirical methods yield nearly identical average distances and fluctuation ranges. For reactants, both methods give S–H $\approx 1.35 \text{ \AA}$ and N–H $\approx 2.35 \text{ \AA}$; in the transition state, S–H and N–H both lie around $1.55\text{--}1.60 \text{ \AA}$;

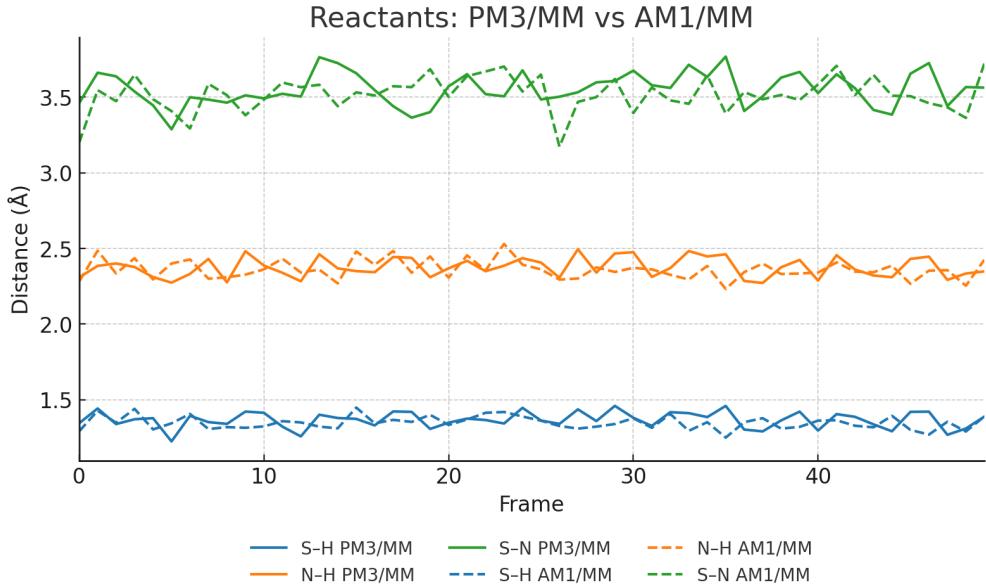


Figure 6: Reactants: PM3/MM vs AM1/MM

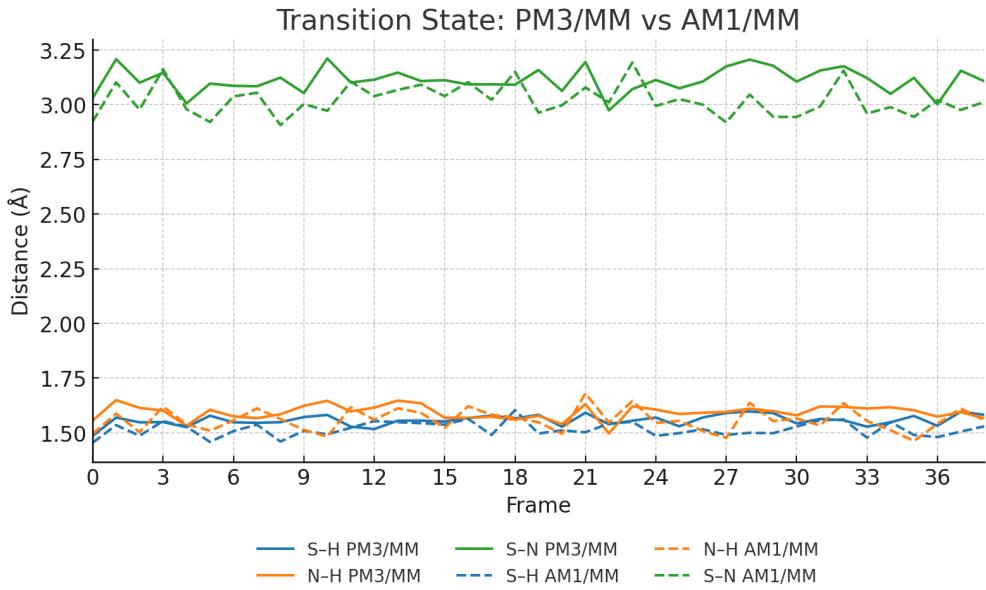


Figure 7: Transition State: PM3/MM vs AM1/MM

and for products, both predict $S-H \approx 1.75\text{\AA}$ and $N-H \approx 1.15\text{\AA}$. The $S-N$ distances likewise match closely in each case. This strong agreement indicates that, despite their different parameterizations, AM1 and PM3 produce equivalent descriptions of the proton transfer geometry and dynamics in the 3CLpro catalytic dyad.

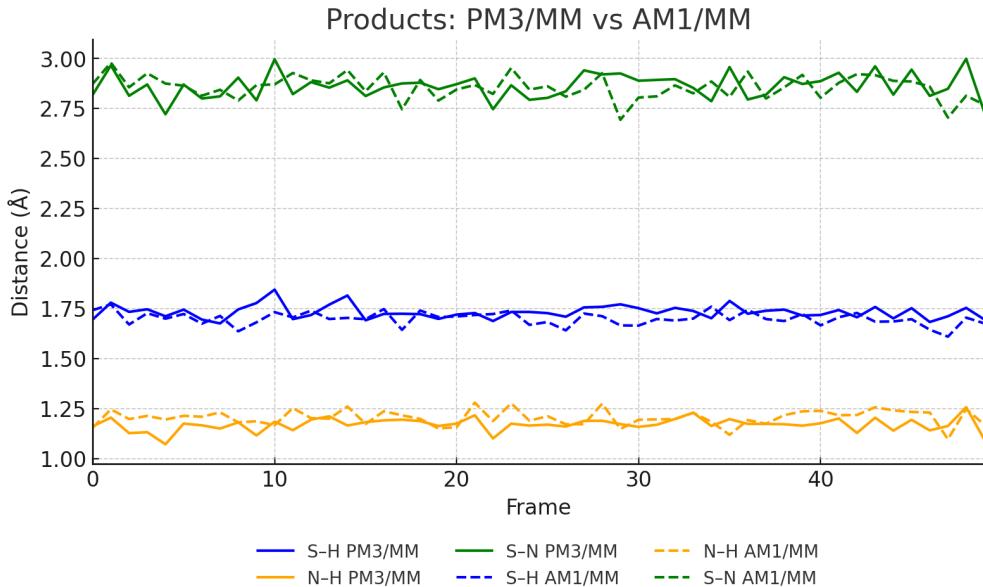


Figure 8: Products: PM3/MM vs AM1/MM

3 Conclusions

In this study, 5ps QM/MM molecular dynamics simulations were performed at three key points along the proton-transfer coordinate of the SARS-CoV-2 3CL protease catalytic dyad—reactants, transition state and products, they revealed clear structural signatures of proton relocation from Cys145 to His41. Distance analyses showed that S–H remains short and N–H long in the reactants, both converge near 1.6 Å in the transition state, and then invert in the products, confirming full proton transfer. AM1/MM and PM3/MM yielded almost identical distance profiles in all three states, demonstrating that both methods provide consistent descriptions of the proton-transfer mechanism.

References

- [1] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, and J. J. P. Stewart, *J. Am. Chem. Soc.* **107**, 3902–3909 (1985).
- [2] J. J. P. Stewart, *J. Comput. Chem.* **10**, 209–220 (1989).