

New Insights into Hydrogen Tunneling in Soybean Lipoxygenase-1 using Quantum Wavepacket *Ab Initio* Molecular Dynamics

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Abstract

We study the hydrogen tunneling problem in soybean lipoxygenase-1 using quantum wavepacket dynamics under the influence of a dynamical active site. The kinetic isotope effect is directly computed using the wavepacket transmission amplitude and the experimental value is reproduced. We find a new insight for the role of the active site geometry during the tunneling process. The changing active site geometry causes a sequence of projections that drives a donor localized wavepacket onto an orthogonal state localized on the product side. This process has connections with quantum measurement theory.

Introduction

Many examples where hydrogen tunneling might play a critical role in enzyme catalyzed biological reactions can be found in the literature. [1] Evidence supporting this hypothesis includes:[1]

- an unexpectedly large primary kinetic isotope effects (KIE= k_H/k_D)
- a weak temperature dependence of the rate constant in conjunction with large KIE
- an elevated value for the Swain-Schaad exponent [$SSE=\log(k_H/k_D)/\log(k_D/k_T)$].

Since these observations are difficult to explain using standard, Arrhenius-type models, tunneling is invoked. We investigate the abstraction of hydrogen from linoleic acid by the non-heme, Fe^{3+} -OH active site complex in soybean lipoxygenase-1 (SLO-1).[1] This reaction is the rate-determining step in the catalytic cycle of SLO-1, which oxidizes unsaturated fatty acids. The room temperature kinetic isotope effect (KIE) for this process is $k_H/k_D = 81$ [1], strongly suggesting a tunneling mechanism. An analysis of the transferred atom with rigorous quantum wavepacket dynamics in conjunction with the simultaneous change in the active site geometry reveals evidence supporting hydrogen tunneling and reproduces the experimental KIE. We also gain a new insight in regards to the role the active site plays in facilitating tunneling. We find that the classical active site behaves like a "measurement device" that acts on the quantum hydrogen. A discrete sequence of these measurements progressively increases the probability of finding the quantum hydrogen in a product state. Although the concept of measurement driven quantum evolution[3] is well-known, **we observe the effect of measurement driven evolution on hydrogen tunneling in enzymes for the first time.**

Active Site and Wavepacket Dynamics

As a model for SLO-1, we excised the active site, an octahedral $Fe(III)$ complex, and truncated the amino acid ligands (see Figure 1). We also used heptadiene to model the substrate, linoleic acid (see Figure 3).

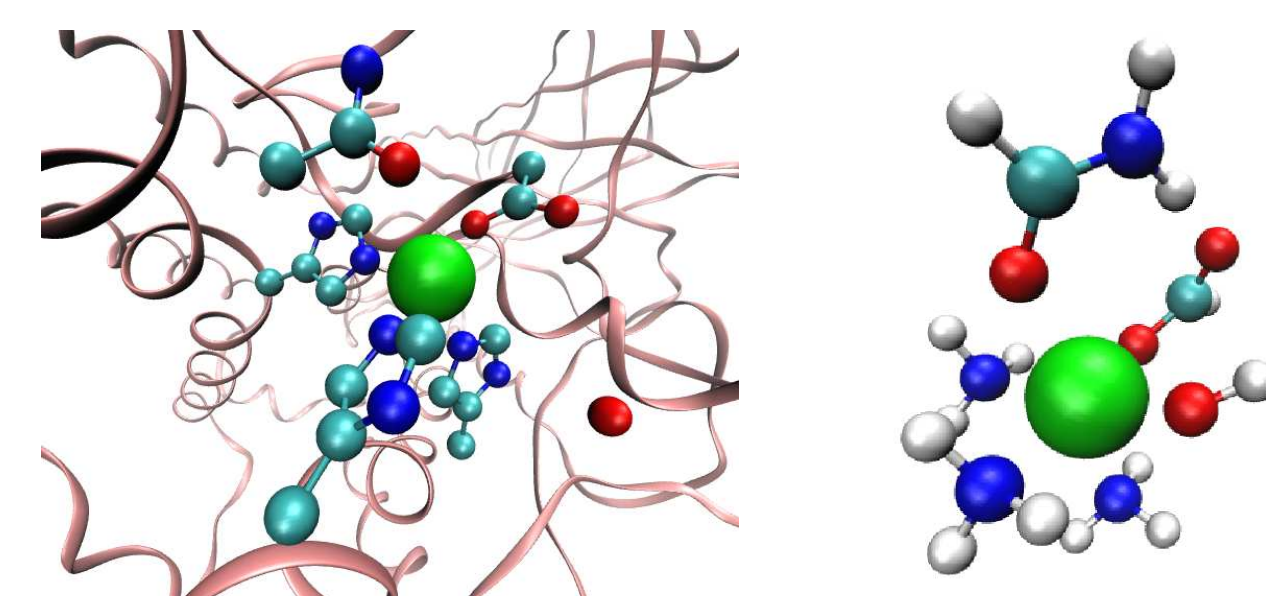


FIGURE 1: The left figure shows the active site embedded in the full enzyme and the right figure displays the model system.

In order to calculate a reaction pathway, we:

- performed a relaxed scan along the reaction coordinate defined as $(R_{CH} - R_{OH})/R_{CO}$, where C is the donor carbon, O is the acceptor oxygen and H is the shared hydrogen
- calculated the tunneling hydrogen potential energy surface at each enzyme-substrate geometry at each step on the reaction coordinate

The potential energy surfaces and optimizations at each step were calculated with B3LYP/LANL2DZ. Each surface is a smooth double well, with the donor side minimum increasing and the acceptor side minimum decreasing in energy along the profile shown in Figure 2. This technique approximates the classical dynamics of the enzyme active site.

The time-evolution of the quantum wavepacket is obtained through Trotter symmetric factorization of the quantum propagator,

$$\chi(x_i, t) = \sum_j \exp\left\{-\frac{iV(x_i, t)t}{2\hbar}\right\} \tilde{K}(x_i, x_j, t) \exp\left\{-\frac{iV(x_j, t)t}{2\hbar}\right\} \chi(x_j, 0) \quad (1)$$

and the free propagator, $\tilde{K}(x_i, x_j, t)$, is approximated [4, 7] using

$$\tilde{K}(x_i, x_j, t) = \frac{1}{\sigma(0)} \exp\left\{-\frac{(x_i - x_j)^2}{2\sigma(t)^2}\right\} \sum_{n=0}^{M/2} \left(\frac{\sigma(0)}{\sigma(t)}\right)^{2n+1} \left(\frac{-1}{4}\right)^n \frac{1}{n!} (2\pi)^{-1/2} H_{2n}\left(\frac{x_i - x_j}{\sqrt{2}\sigma(t)}\right) \quad (2)$$

The initial wavepacket is constructed as a Boltzmann average of the energy eigenstates at time 0. Using Eqs. 2 and 1 the wavepacket is propagated on the changing potential energy surfaces described above. Thus, the wavepacket evolution is influenced by the active site evolution.[7, 6].

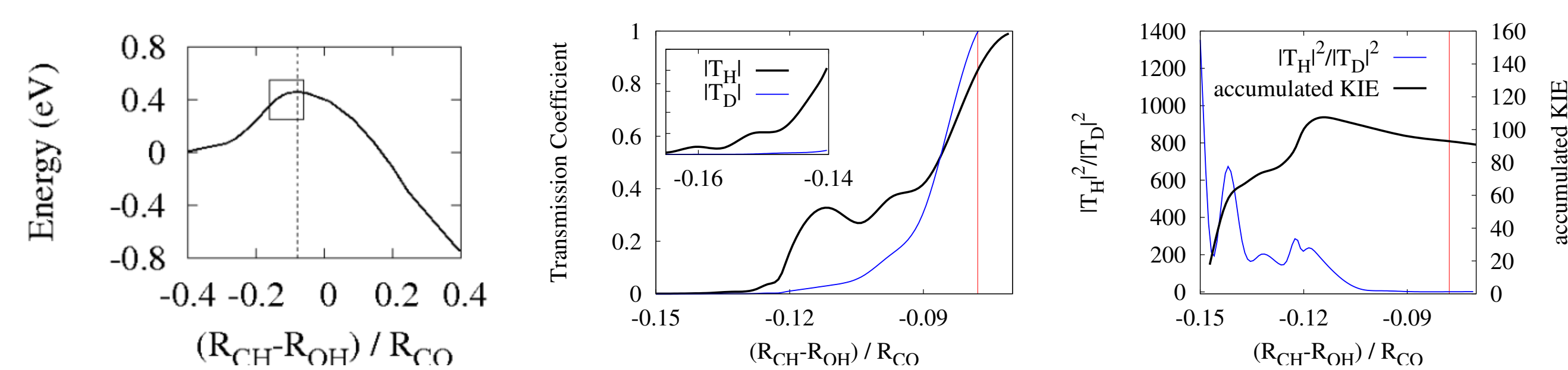


FIGURE 2: The left figure shows the reaction profile with the transition state region highlighted with a box. The middle and right pictures magnify this region and display the transmission coefficients and the accumulated KIE. The classical transition state is marked with a vertical line in all figures.

The total probability of the wavepacket on the product side leads to the transmission coefficients shown in Figure 2. The transmission coefficients for hydrogen and deuterium are significant before the classical barrier maximum, indicating tunneling in both cases. Since the transmission coefficient corresponds to the population in the product state, the ratio shown in Figure 2, can be used to directly obtain the KIE. **No pre-factors are used to obtain the KIE since the energetic factors are already present in the wavepacket dynamics.** In Figure 2, the KIE converges to an average of 88, in good agreement with experiment[1].

Eigenstate Evolution

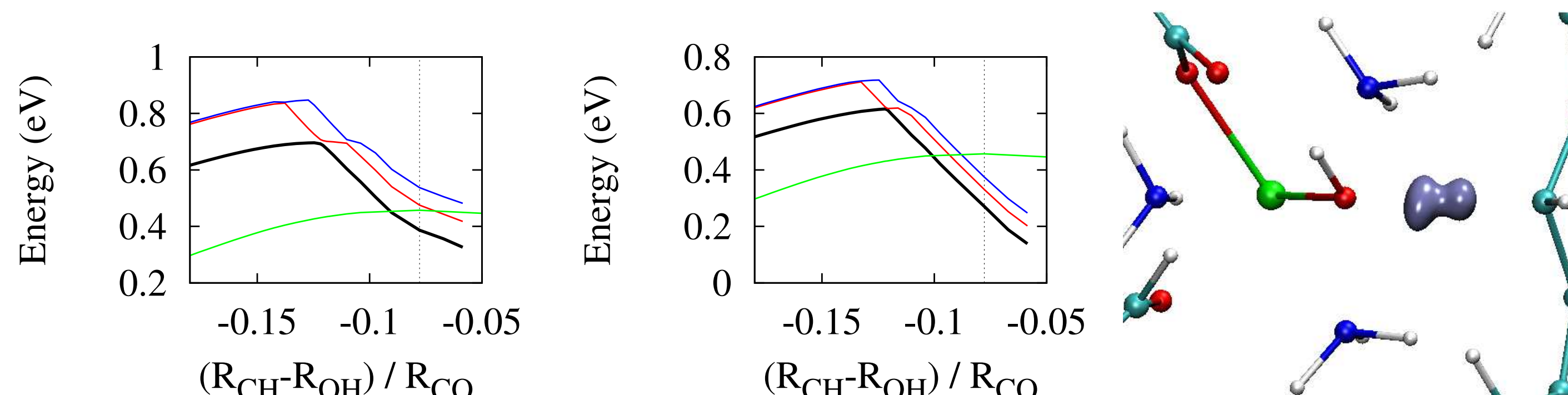


FIGURE 3: The left and middle figures depict the evolution of the eigenenergies in the critical tunneling region. The far right figure shows the ground eigenstate at the "quantum" transition state $(R_{CH} - R_{OH})/R_{CO} = -0.12$

Eigenvalues for each Hamiltonian along the reaction coordinate were calculated using an efficient Arnoldi iterative diagonalization scheme.[6] Figure 3 depicts the evolution of the hydrogen and deuterium energy eigenvalues along the reaction coordinate. Clearly, the transition state shifts to the left as a result of the nuclear quantization. Although this effect has been noted before using variational transition state theory[2], we emphasize **that we show these effects purely based on the nuclear quantization and associated potential energy surfaces.** We should also note that the eigenvalue energies dip below the reaction profile, since they are localized on the product side. The active site geometry and hydrogen ground state at the "quantum" transition state is at the right in Figure 3. Finally, in Figures 4 and 5, we depict the evolution of the hydrogen and deuterium ground states at different points along the reaction profile.

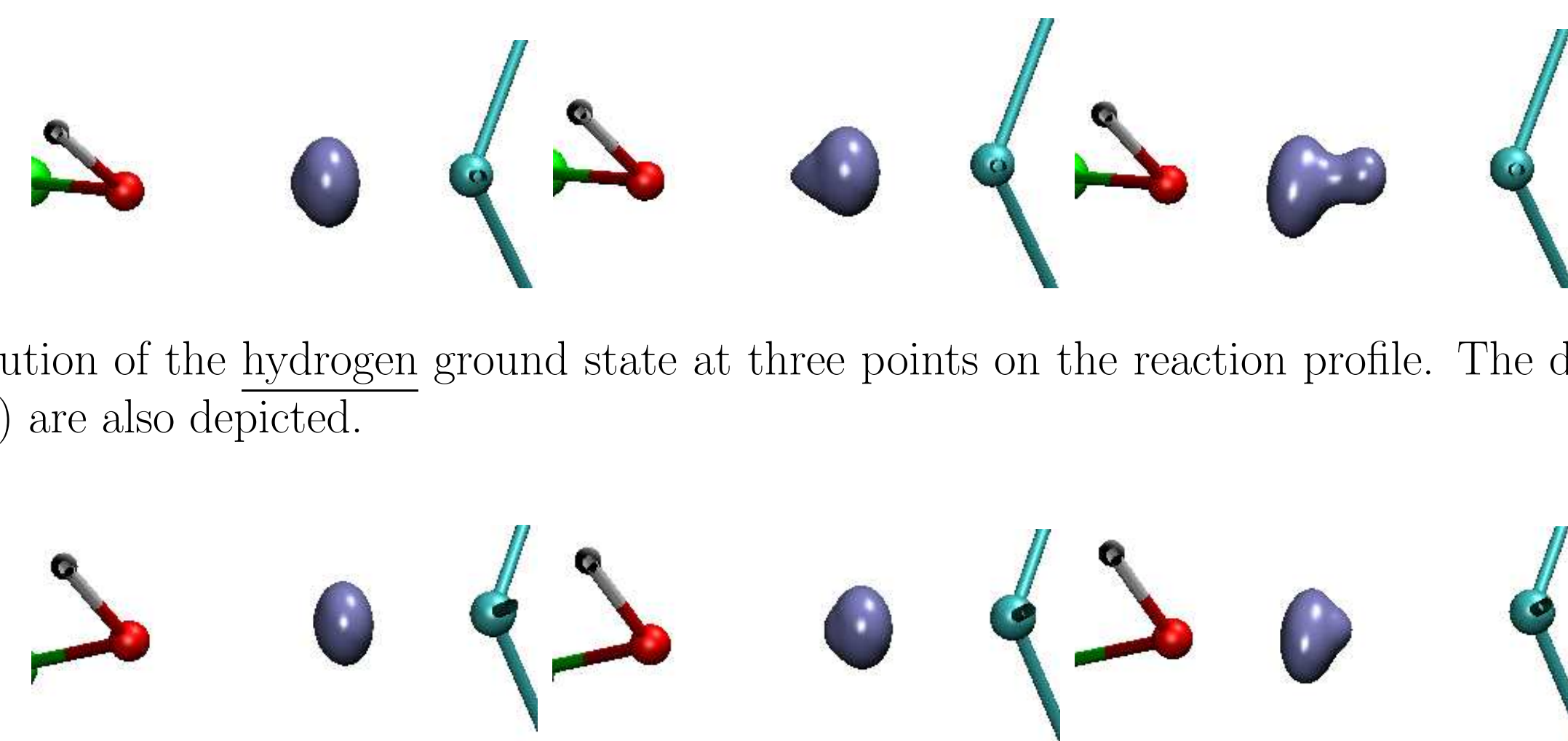


FIGURE 4: The evolution of the hydrogen ground state at three points on the reaction profile. The donor carbon (right) and acceptor oxygen (left) are also depicted.

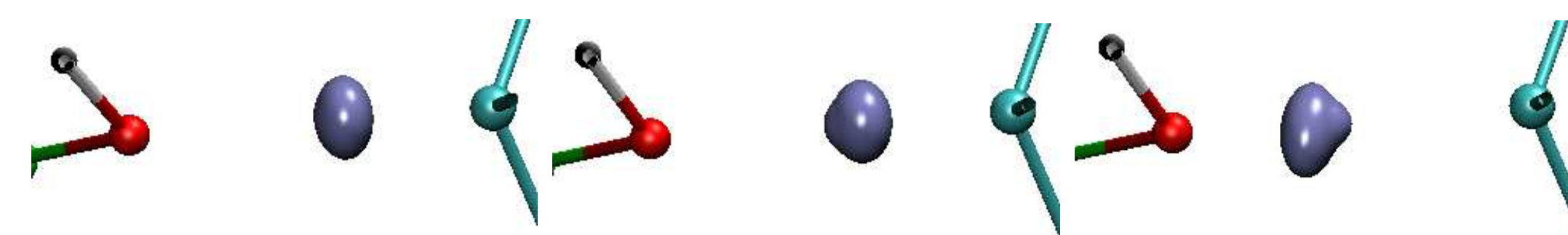


FIGURE 5: The evolution of the deuterium ground state at three points along the reaction profile. The donor carbon (right) and acceptor oxygen (left) are also depicted.

We also calculated the contributions of the eigenstates to the time-dependent wavepacket at points along the reaction coordinate. It is interesting to note that near the barrier, excited states play an important role, more so for deuterium. This observation might explain the temperature dependence of the KIE in SLO-1.

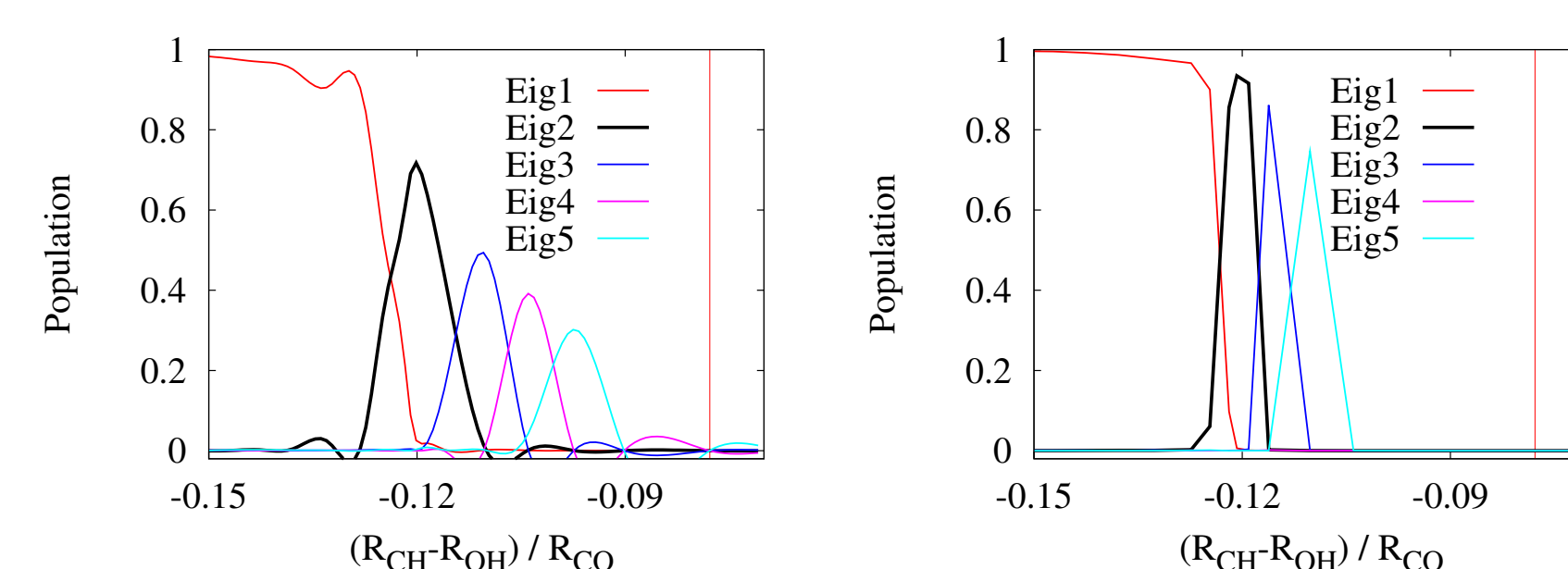


FIGURE 6: The instantaneous wavepacket components along the time-dependent eigenstates. Hydrogen is depicted on the left, and deuterium on the right.

Connections to Measurement Theory

In SLO-1, the active site geometry and the hydrogen wavepacket interact as they simultaneously evolve. What is the nature of the interaction between the two subsystems (classical active site and quantum wavepacket) and does it facilitate tunneling? In this section, we argue the following points:

- measurements can be used to drive a quantum system from one orthogonal state to another [3]
- the dynamical active site interacts with the quantum hydrogen in SLO-1 as if it were performing measurements on the quantum system at each time step[5]
- the active site "measures" the quantum state and drives it from donor to acceptor

In quantum mechanics, a measurement is a projection of the input state onto a complete set of eigenstates of the measurement operator. A measurement, M , can affect the state of a system whenever the input state is not an eigenstate of M (for example, $M|O\rangle = |O'\rangle$ changes the input state and $M|M\rangle = m|M\rangle$ leaves the state unperturbed). In our case, the quantum proton is exposed to a sequence of Hamiltonians that change with the active site geometry during the reaction. Thus, the evolving quantum state may be projected onto a new set of basis vectors (eigenstates of time-dependent active site Hamiltonians) at every instant. To better understand this argument, consider a two-dimensional Hilbert space with orthogonal kets $|D\rangle$ and $|A\rangle$. In this case, $|D\rangle$ represents a state localized on the donor, $|A\rangle$ represents a state localized on the acceptor side and $|I_i\rangle$ represent the eigenstates of a series of intermediate measurements. Imagine the following process:

- at time t , the quantum wavepacket is in a donor state $|D\rangle$
- at time $t + \Delta t$, the state is changed via an interaction with a measurement device with eigenkets $|I_i\rangle$ (recall $\hat{M}_i|D\rangle = |D'_i\rangle$)
- the new state now has a non-zero projection on the product side (the probability of being in the product state, $|\langle D'_i|A\rangle|^2$, is no longer zero)
- at time $t + 2\Delta t$, a different measurement on the state $|D'_i\rangle$ changes the probability of being on the product state once again
- this measurement process allows the quantum hydrogen in SLO-1 to evolve from a donor to an orthogonal acceptor state

In our case, the measurement device is the classical active site and the measurement operator is the system Hamiltonian, $H(t)$. Since the active site evolves, the Hamiltonians and its eigenstates change at each time step. For the two dimensional Hilbert space, it can be shown that the projection of the quantum state onto $|A\rangle$ after a sequence of N non-commuting measurements is

$$\left\{1 - \prod_i \left[1 - 1/2 \sin^2 2\theta_i\right]\right\}, \quad (3)$$

where $\cos \theta_i = \langle I_i|D\rangle$. Thus, θ_i represents the angle between eigenstates corresponding to the i -th measurement with the donor state. In addition, it can be shown that the commutator of the two measurement operators which have eigenstates differing by an angle θ , is also proportional to $\sin^2 2\theta$, an observation that becomes important later. The analogy between this two-dimensional Hilbert space and the proton tunneling problem considered here is as follows:

- the initial donor state is sequentially projected at every instant onto an eigenstate of the Hamiltonian
- this sequential projection (measurement) drives the quantum evolution of the tunneling particle from the donor state to the orthogonal acceptor state.

Let us now consider two sequences of measurements with angles $\{\theta_i\}$ and $\{\phi_i\}$ in Eq. (3). Let us further assume that in the sequence $\{\theta_i\}$, $|\theta_{i+1} - \theta_i|$ monotonically increases but $\{\phi_i\}$ does not. It is apparent that the projection in Eq. (3) effected by the sequence $\{\theta_i\}$ grows faster than that effected by $\{\phi_i\}$, for small difference in the subsequent angles. Since the angles relate to the commutators of measurement operators, a growing sequence of commutators is liable to provide a larger projection on the acceptor state like a growing sequence of angles in Eq. (3). Can we observe such a phenomenon in our simulations?

To determine the relationship between Hamiltonians at different times, we compute a measure of the Boltzmanized commutator, $[H(t + \Delta t), H(t)]_\beta$, in Figure 7:

$$\left\| [H(t + \Delta t), H(t)]_\beta \right\| \equiv \left\| \left[\sum_i \exp[-\beta \epsilon_i^{(t+\Delta t)}] \epsilon_i^{(t+\Delta t)} |\phi_i(t + \Delta t)\rangle \langle \phi_i(t + \Delta t)|, \sum_i \exp[-\beta \epsilon_i^{(t)}] \epsilon_i^{(t)} |\phi_i(t)\rangle \langle \phi_i(t)| \right] \right\|, \quad (4)$$

The quantity β is the inverse temperature. Since this quantity grows monotonically with $|T_H|$ but not with $|T_D|$ (Figure 7), these results, seem to suggest that **the active site dynamics increase the tunneling probability during the reaction.**

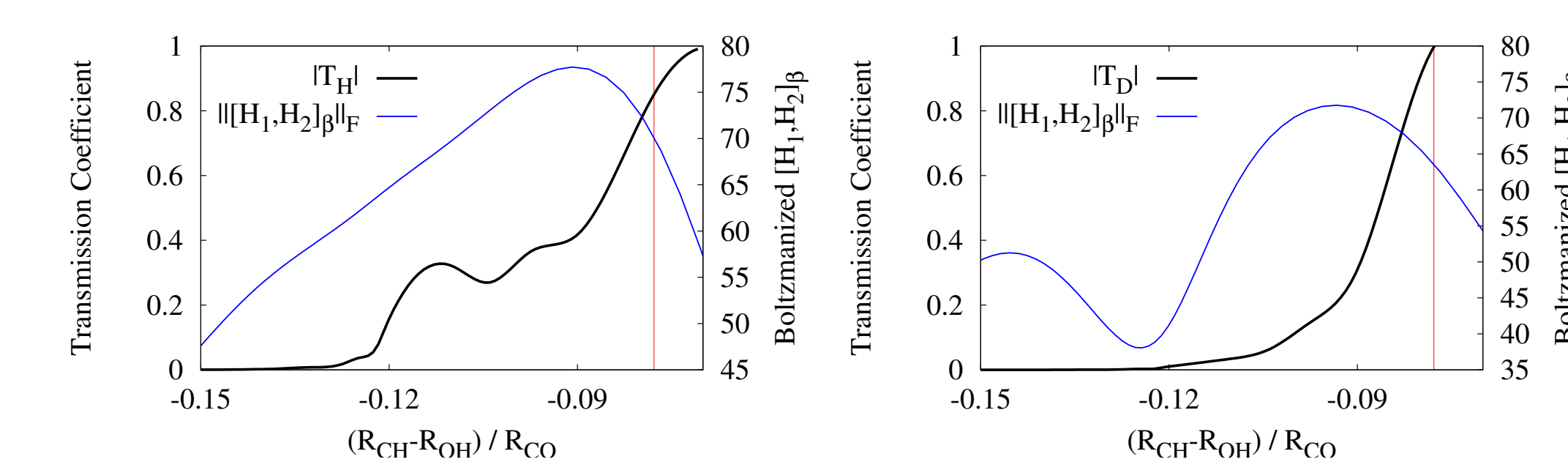


FIGURE 7: Norm of the Boltzmanized commutator is compared with the transmission coefficient for hydrogen (left) and deuterium (right).

While the above analysis pertains to a two-dimensional Hilbert space, it is important to note that the Boltzmann weight for the first two proton and deuteron eigenstates dominate across the reaction coordinate. Furthermore, as seen in Figures 7, after the wavepacket reaches the classical transition state, it has a high average kinetic energy which does not allow the active site atoms to move quickly between time-slices and hence, the commutator decreases monotonically in absolute value.

Conclusions

In summary, we have:

- created a reaction coordinate for SLO-1 and generated a hydrogen potential energy surface at points along the profile
- propagated a quantum wavepacket with a time-dependent Hamiltonian (time-dependent potential)
- uncovered evidence for tunneling, in particular we have shown that the transition state shifts towards the reactant solely as a result of quantization
- reproduced the experimental KIE for SLO-1 without any correction factors using wavepacket propagation
- connected the tunneling process to measurement theory, where the active site geometry acts as a measuring device that causes the quantum state to evolve from a donor to acceptor state.
- shown that $[H(t + \Delta t), H(t)]_\beta$ increases along with the wavepacket transmission coefficient along the reaction profile, suggesting the active site dynamics enhance tunneling during the reaction

We believe that the reproduction of the experimental KIE without using corrections to classical expressions is an exciting validation of the methodology presented here. We also believe that the connection to measurement theory, along with the growing sequence, $[H(t + \Delta t), H(t)]_\beta$, may be fundamental. Future studies will be geared towards studying the amino acid dynamics that cause the commutators to grow and further investigations into the active site measurement of the quantum subsystem will also be conducted.

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