

Response to Comment on “Effect of Active Site Mutation Phe93 → Trp in the Horse Liver Alcohol Dehydrogenase Enzyme on Catalysis: A Molecular Dynamics Study”

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Warshel and Villà-Freixa have commented on our manuscript. There are two main claims in their manuscript. First, they claim that their work and earlier work of one of them demonstrate that there is no role played by dynamics in enzymatic catalysis. Second, they express consternation that not enough credit has been given to Warshel and co-workers for their contributions to this field. We now discuss each issue in turn.

Warshel and Villà-Freixa state clearly that their computations show that dynamics plays no role in enzymatic catalysis. For example, their viewpoint was cogently explained in the pages of this journal.¹ As they state in this review: “*Thus if we can estimate the rate constant by Monte Carlo approaches, we do not have dynamical effects.*” It is generally accepted that any dynamical effects on the rate must lie in the transmission coefficient κ . The main question, therefore, is whether κ is much greater in some enzymatic reactions than in the corresponding reaction in solution.” (Italics are from Villa and Warshel.) We will not comment on the methodologies they use to estimate the transmission coefficient though there are many technical issues related to their approach. Rather we will present a simple counterexample showing that their logic regarding the use of κ as a diagnostic of potential contributions of enzymatic dynamics to catalysis is faulty.

Consider a two-dimensional potential energy surface for a simple gas-phase chemical reaction. We imagine the reactants and products to be symmetrically placed with regard to the saddle point on the potential surface. As a classic example, consider the $\text{H} + \text{H}_2$ reaction—the archetype of such computations. As Pechukas demonstrated many years ago,² transition-state theory for such symmetric reactions is in fact exact (within a certain energy distance from the top of the barrier.) Thus by definition, $\kappa = 1$. Now let us consider the same potential energy to which we add a symmetrically coupled vibration. As we have shown,³ the symmetric coupling shifts the position of the minima and the height of the barrier but not the overall symmetry of

the transition state with respect to reactants and products. In the language of gas-phase reaction rate theory, the curvature of the reaction path is increased, the barrier is modulated in width and height, but the symmetry is maintained. In this case, κ is again exactly equal to 1. Using the criterion of Warshel and co-workers, one would conclude that there was no dynamic effect of the symmetrically coupled vibration on reaction. It is clear, however, that periodic modulation of the barrier affects rate. We note that Warshel makes reference to the work of Hammes-Schiffer et al.⁴ and states that they also believe that dynamics in the enzyme horse liver alcohol dehydrogenase (HLADH) plays no role in catalysis. They clearly also *define* dynamics contributions in terms of the transmission coefficient. In fact, given the symmetric nature of the coupling, we would be quite surprised if there was an effect on the transmission coefficient, for the reasons delineated by Pechukas 25 years ago.

There is a further difficulty with the logic in Warshel’s critique. Inherent in the argument is the curious viewpoint that statistical quantities such as free energies of activation can inform directly on dynamics. While it is true that dynamics are needed to compute these quantities, the converse is clearly not true—statistical quantities themselves do not imply knowledge of how dynamics affects rate. It is likely that the effects can be reproduced by thermal averages—the types of motion that we have identified are quite fast—and an adiabatic separation is probably valid. This however begs the interesting mechanistic question.

The second major point to the comment by Warshel was an extended complaint that too much credit was given in our manuscript to other investigators and not enough to Warshel’s group. For example, it is pointed out that “The paper by KS starts with an apparent misunderstanding of current proposals. This includes attribution to Pauling of the idea that when the reactants bind to enzymes they are close to the top of the uncatalyzed reaction barrier.” It is curious that they take such umbrage at this attribution, given that in their own review they state “The fact that the activation barrier is reduced by the enzyme was stated by Pauling long ago.” Whatever their difficulty with our attribution of the idea of transition-state stabilization to Pauling, we state without reservation that the contributions of the Warshel group are many.

References and Notes

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