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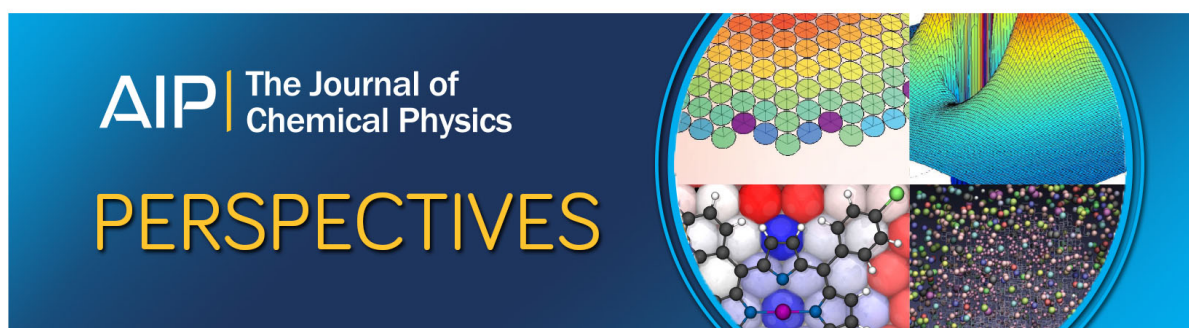
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Reaction path determination for quantum mechanical/molecular mechanical modeling of enzyme reactions by combining first order and second order “chain-of-replicas” methods

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A two-step procedure for the determination of reaction paths in enzyme systems is presented. This procedure combines two chain-of-states methods: a quantum mechanical/molecular mechanical (QM/MM) implementation of the nudged elastic band (NEB) method and a second order parallel path optimizer method both recently developed in our laboratory. In the first step, a reaction path determination is performed with the NEB method, along with a restrained minimization procedure for the MM environment to obtain a first approximation to the reaction path. In the second step, the calculated path is refined with the parallel path optimizer method. By combining these two methods the reaction paths are determined accurately, and in addition, the number of path optimization iterations are significantly reduced. This procedure is tested by calculating both steps of the isomerization of 2-oxo-4-hexenedioate by 4-oxalocrotonate tautomerase, which have been previously determined by our group. The calculated paths agree with the previously reported results and we obtain a reduction of 45%–55% in the number of path optimization cycles. © 2005 American Institute of Physics. [DOI: 10.1063/1.1860560]

I. INTRODUCTION

Enzymes play a critical role in biological systems because of their catalytic ability. A key question in understanding enzymatic catalysis is which mechanism enzymes use to carry out their respective chemical reactions. Theoretical investigation of the reaction mechanisms for enzyme systems has been a field of intense study for some time.^{1–6}

The theoretical calculation of an enzyme mechanism involves the determination of the reaction path, as well as the free energies of activation associated with this path. Ideally, in order to determine reaction mechanisms rigorously, a large number of paths need to be sampled and considered as an ensemble.⁷ For enzyme reactions, however, the sampling of multiple reaction paths is computationally unfeasible because of the size of the systems.

In the case of enzymatic systems, generally one, or at most a few minimum energy paths (MEPs) are considered to be representative. The MEP is defined as the steepest descent path that connects two minima (reactant and product) with a first order saddle point (transition state). In some instances, a couple of enzyme-substrate conformations are generated to sample the path ensemble, and the MEP is optimized for each conformation.⁸ Once the MEP has been determined, it is possible to improve the description of the catalytic mechanism. This may be accomplished by calculating the free energy changes associated with the reactions, as well as the dynamic effects of barrier recrossings.^{6,9–11}

Recently, several methods have been developed by our group for the determination of enzymatic reaction mechanisms. For the determination of MEPs in enzymes we have adapted three methods: the reaction coordinate driving method (RCDM),^{9,12} the nudged elastic band (NEB) method,^{13–18} and a second order parallel path optimizer.^{19,20}

In order to go beyond the MEP, we have also developed a free energy perturbation (FEP) method to determine the change in free energy along the reaction coordinate.⁹ To improve this FEP procedure, we developed a novel reaction path potential (RPP) model for the calculation of reaction dynamics in *ab initio* quantum mechanical molecular mechanical (QM/MM) potentials.¹⁰ Furthermore, we have applied the RPP model to enable the calculation of transmission coefficients.¹¹

In the present contribution we will concentrate on the efficient determination of the MEPs associated with enzymatic reactions. As explained above, several methods have been developed for the determination of MEPs such as RCDM, NEB, and the second order parallel path optimizer.

The determination of MEPs with the RCDM method is challenging. In these calculations a reaction coordinate is chosen along which the system is constrained and gradually modified from reactant to product. Although this method is one of the most chemically intuitive, there are many disadvantages. For example, in many cases the selection of a reaction coordinate may not be straightforward and many re-

action coordinates may need to be sampled. The initial path obtained with RCDM may be discontinuous and several reaction driving calculations both in the forward and backward directions may be necessary, which increases the computing time. Furthermore, the implementation of a parallel computing scheme for this method is very difficult because of its stepwise nature.

The parallel path optimizer and NEB methods are types of “chain-of-replicas” method.^{13,14,18–22} In these methods the MEPs are represented by a discrete number of structures that form a chain connecting the reactant to the product. In general, the initial intermediate structures are obtained by a linear interpolation between the end points, and subsequently all points are optimized to the MEP simultaneously. This parallel optimization is possible since any point on the MEP is a minimum in all directions except for the reaction coordinate and thus the energy gradient for any point is parallel to the local tangent of the reaction path.

The parallel path optimizer method has been recently developed by our group for the study of MEPs in enzyme reactions. This method was developed by extending the procedure of Ayala and Schlegel for small molecules.¹⁹ It is a second order method which combines synchronous transit and quasi-Newton methods. Here the path is also represented by a set of discrete points connecting the reactant and product. For each point the total energies and gradients are calculated and empirical Hessians are obtained. The highest energy point is chosen to optimize to the closest transition state (TS) which divides the path into two downhill segments. If the end points are not already at a minimum, they can be optimized to the closest minimum. The remaining points on the path are optimized to lie on the steepest descent path.

NEB is a first order method in which the images are connected by a spring interaction force which ensures equal spacing of the replicas along the path. At each step the energy and gradient of each point are calculated and the tangents along the reaction path are determined. These energies and gradients are employed to optimize the points on the path by minimizing the forces acting on the images, which results in the chain being optimized to the MEP.

The main advantage of the NEB method to calculate MEPs for enzymes is that it shows fast initial convergence. It is also able to handle reaction mechanisms with multiple steps and multiple intermediates. However, the main disadvantage of this method is that it does not have an explicit TS finder and its convergence is only of first order. To overcome this obstacle an adaptive NEB method has been proposed.¹⁶ An alternative method is to employ a large number of replicas to sample the path.¹⁵ However, in both cases the computing time is significantly increased.

On the other hand, the parallel path optimizer method has the advantage of a true TS optimizer. In addition, this is a second order method, and thus has a faster convergence for paths close to the quadratic region. However, this method presents slow convergence when the initial guess to the path is far from the MEP. Another disadvantage of this method is that it can only study single step reaction mechanisms.²⁰ This

method may be extended to handle multiple steps, however this would require previous knowledge of the reaction mechanism, which renders it impractical.

Therefore, we develop here a procedure that makes use of both methods in order to take advantage of their strengths while avoiding their respective weaknesses. We start by performing an NEB calculation for the path with loose convergence criteria in order to obtain a “rough guess” to the MEP. During this portion of the procedure, we employ an iterative restrained MM optimization as developed by Xie *et al.*¹⁸ Subsequently, this rough guess is employed as a starting point for the parallel path optimizer method calculation to obtain the converged MEP, which includes the optimized TS.

II. METHODS

We now proceed to the description of the combined procedure for MEP determination and the methods included in this procedure. This section is organized as follows: in Secs. II A–II D we provide a brief overview of the methods employed in the combined procedure including the NEB and parallel path optimizer methods. Subsequently, in Sec. II E we provide the details of the new development, namely, the combination of these methods for the determination of MEPs in enzyme systems.

A. Iterative QM/MM optimization

All our calculations are performed using QM/MM methodology that includes the pseudobond model for the QM/MM boundary.^{9,23,24} This methodology has been implemented in a modified version of GAUSSIAN 98,²⁵ which interfaces to a modified version of TINKER.²⁶ The AMBER94 all-atom force field parameter set²⁷ and the TIP3P²⁸ model for water are used.

Path optimizations are carried out by an iterative minimization procedure as explained in our previous work.²⁰ Briefly, because of the large number of degrees of freedom associated with these systems we partition them into a core set and an environmental set. The core set is small and contains all the degrees of freedom that are involved with the chemical steps of the reaction, while all the remaining degrees of freedom are included in the environmental set. In all the QM/MM calculations presented in the results section, the core set is defined by the QM subsystem and the environmental set by the MM subsystem.

During the optimization of the MEP only the degrees of freedom in the core set are used to define the tangents parallel to the path. This ensures that the floppy degrees of freedom from the environmental set do not interfere in the optimization by satisfying the equal-spacing constraints enforced on the replicas, which may be easily satisfied by large fluctuations of the environmental set that could be unrelated to the reaction path. These floppy degrees of freedom present an additional challenge since it must be ensured that the reorganization of these degrees of freedom during the optimization procedure are associated with the reaction; otherwise, the changes in total energy along the path cannot be associated with the reaction because they include fluctuations that are irrelevant to the reaction process.

B. Nudged elastic band method

NEB is an efficient chain-of-replicas method for finding MEPs. Here the path is simulated by an elastic band with $N+1$ images. In this case the end points x_0 and x_N are fixed and correspond to the reactant and product of the system under study. The $N-1$ remaining images are optimized in Cartesian coordinates using a projected velocity Verlet algorithm by minimizing the forces acting on the images along the path.¹³ A spring interaction is included between the images to ensure equal spacing along the path. A unique feature of NEB is that the forces are projected in such a way that the spring forces do not interfere with the minimization. The projection of the forces also ensures that the true forces do not affect the image distribution along the MEP.

In order to perform the NEB calculations on enzymes in the present contribution, we have interfaced a parallel NEB method²⁹ with the QM/MM software developed in our lab.^{9,25,26} In this way, the energies and forces required by NEB to optimize the core set of degrees of freedom for the points lying on the path are calculated with our QM/MM methodology. This implementation of NEB includes the ability to input intermediate structures in addition to the end point structures for the linear interpolation. This feature allows a more efficient determination of the MEP if certain intermediates are known in advance.

We have also included an improved tangent estimate as proposed by Henkelman and Jónsson.¹⁵ This ensures that there will be no kinks on the band where the parallel component of the force is large compared with the perpendicular component, generally found near the inflection points of the energy curve for the MEP.

C. Restrained environment optimization

As explained above, the initial path is represented by a fixed number of replicas of the system which are obtained by a linear interpolation of the core set between the reactant and the product. To ensure a smooth and continuous change of the environment set during the optimization, the initial coordinates for the environment atoms are set to be the same for all the replicas along the path. Thus, the initially guessed replicas differ only in the coordinates of the core atoms. In other words, the initial environment for all the points on the path is the same and is taken to be the environment from one of the end points.

The path is iteratively minimized, starting with the concerted optimization of the core set of atoms (path optimization) with either the NEB or the parallel path optimizer method, followed by the optimization of the environment set of atoms in Cartesian coordinates with a truncated Newton method. During the optimization of the environment, the path may become discontinuous in the early cycles because the initial guess to the MEP may not be accurate. However, this problem can be overcome by performing a restrained minimization of the environment set as developed by Xie *et al.*¹⁸ In this method, the environmental atoms are restrained during the MM optimization. These restraints are gradually

reduced after each cycle until no restraints remain, ensuring a smooth change in the MM environment and avoiding sudden fluctuations.

D. Parallel path optimizer method

In this method the reaction path is refined until the transition state and steepest descent path are found, starting from an initial path or a previously determined path. The highest energy point on the path is optimized to the closest TS while the end points are optimized to the closest minima and the intermediate points are optimized to lie on the steepest descent path. At each step in the optimization the energy and gradient of every point on the path is calculated and an empirical Hessian is obtained.

The optimization of the core set of the points is performed in redundant internal coordinates and is guided by its immediate neighbors. To avoid rigid body rotations between the core and environment set the Cartesian coordinates of at least three atoms in the core set are included in the redundant internal coordinate set.²⁰

The TS, described by the coordinate \mathbf{x}_j^c , is optimized by assuming that the transition vector is tangent to the arc of circle that passes through the \mathbf{x}_{j-1}^c , \mathbf{x}_j^c , and \mathbf{x}_{j+1}^c points. This tangent is used to guide the quasi-Newton/eigenvector following optimization of the TS. For the intermediate points that lie on the steepest descent portions of the path, a modified Gonzalez–Schlegel path following method³⁰ is used. At each point the segment connecting a point \mathbf{x}_i^c to its uphill neighbor \mathbf{x}_{i+1}^c (\mathbf{x}_{i-1}^c in Ayala and Schlegel¹⁹) is approximated by an arc of circle defined by these two points and the tangent to the path τ_{i+1} . For each segment a local quadratic surface about each point is used to relax that point so that the path differential is satisfied.

During the course of the optimization step, an extra spacing constraint is imposed on the intermediate points between the TS and the minima on each side of the path. This extra constraint results in equal spacing between all the points along the path. The equations for the path relaxation and spacing constraints are solved in a series of microiterations until the convergence criteria are met or the trust radius is exceeded.

The quality of the Hessian is critical for this procedure. Initially the Hessians are empirically estimated for each point and are updated in the following optimization steps using the available gradients. Different Hessian updates are necessary for the points along the path.¹⁹ For the end points a Broyden–Fletcher–Goldfarb–Shanno (BFGS) method is used, for the TS a Bofill update is used, and for the rest of the points a BFGS/Powell mixture is used.

In the QM/MM context, the importance of the Hessian update is increased since the core set of atoms is influenced by the environment set. When environment reorganizations are considered, the core set of atoms in neighboring replicas are in different fields. Since the Hessian update is performed using information from neighboring points, it is very important for the environment to change continuously along the path.

After each path optimization step, the energies and gra-

dients are calculated in parallel and the Hessians are updated using information from the previous steps. This procedure is repeated iteratively until all the points are converged.

E. Combined NEB and parallel path optimizer procedure

We now describe our main development of this paper. The procedure consists of two steps: the optimization of the path with NEB, followed by the optimization of the path with the parallel path optimizer method. Initially, the path is iteratively optimized with the NEB method for the core set followed by an optimization of the environment set. When the core set is optimized with NEB, the convergence is chosen to be relatively loose to reduce the computing time. The optimization for the environment set is carried out in stages with a restrained minimization in which the positions of the active atoms in this set are restrained.¹⁸ The restraints on these atoms are reduced after each iteration until none remain.

Once the path has been optimized with NEB, it is used as the initial guess for the parallel path optimizer method. In this second part, the path is again iteratively optimized. The core set is optimized with the parallel path optimizer method, followed by the optimization of the environment set. In this part of the calculation, the environment set is no longer optimized by restrained optimization. The iterations are continued until all the convergence criteria are met and the final optimized MEP is obtained.

If the path obtained from the NEB procedure has one or more intermediates, the path needs to be partitioned into single step paths. This partition is required since the parallel path optimizer method can only deal with single step reactions. For example, if the path contains one intermediate and two TSs, we can partition the overall path into two segments, both of which contain the intermediate point. These two paths can then be optimized separately to obtain the final overall path.

When both of these single step paths are optimized, the intermediate point from the overall path is taken as the product for the first step. For the second step, the intermediate is taken to be the reactant. The continuity of the path is guaranteed even if the path is separated in this way. The reason is that the parallel path optimizer method performs an unconstrained minimization on the end points, which permits the intermediate point on both paths to optimize to the same minimum. However, the intermediate structure is optimized in both paths, which results in the allocation of an extra computer node for the same point.

Another way to guarantee continuity of the overall path is to optimize the first step path initially to obtain the optimized intermediate structure. After this path is converged, the optimized structure for the intermediate can then be used to optimize the second step path without the need to optimize the intermediate structure again. This method also guarantees continuity of the path without optimizing the same intermediate twice. However, the paths need to be calculated sequentially in order to obtain the optimized structure for the intermediate connecting the different steps along the MEP.

F. Computational procedure

In practice, for the calculations presented in this contribution, in the first part of the procedure several iterative optimization cycles (NEB/MM cycles) were performed. In each cycle the core set was optimized with NEB followed by the optimization of the environment set. After each NEB/MM cycle was completed, the convergence criteria for the core set was reduced. In the first cycle of the NEB optimization, the path was considered converged when the gradient of the core set of atoms for all the points was less than 0.04 a.u. This threshold was reduced by 0.005 a.u. in each cycle without going below 0.01 a.u. for the last stages. The maximum allowed number of path optimization steps per cycle for NEB was set to ten.

In the case of the restrained MM optimization performed after every NEB cycle, all points were required to converge to 0.1 kcal/mol Å². The position of the active atoms in the environment set were restrained with a force of 500 kcal/mol Å² in the initial cycle which was reduced in each subsequent cycle to 200, 100, 50, 25, and 10 kcal/mol Å². In the final cycle an unrestrained minimization was performed to relax the environment.

For the parallel path optimizer method, we employed the same convergence criteria as in our previous work.²⁰ For all the points optimized to stationary points we used the default convergence in GAUSSIAN 98, while for the uphill and downhill points the displacement criteria was loosened to five times the default.

III. CALCULATIONS AND RESULTS

To show the applicability and performance of our procedure we have applied it to 4-oxalocrotonate-tautomerase (4OT). 4OT is a hexameric bacterial enzyme that catalyzes the isomerization of unconjugated α -keto acids such as 2-oxo-4-hexenedioate (2o4hex) to its conjugated isomer 2-oxo-3-hexenedioate (2o3hex). Note that 4OT also catalyzes the isomerization of 2-hydroxymuconate (2HM) to 2o3hex (see Fig. 1), however, under physiological conditions 4OT uses 2o4hex as substrate.

In a previous study³¹ we confirmed the reaction mechanism proposed by Harris *et al.*³² for 4OT. This is known to be a general acid-base reaction that takes place in two steps (see Fig. 1). In the first step, one of the hydrogens from C3 of the substrate (2o4hex) is abstracted by the nitrogen atom of Pro-1. This produces a negative charge on the carbonyl oxygen of the substrate in the transition state and intermediate structures, which is stabilized by Arg-39 and an ordered water in the active site. For the second step the proton is returned from Pro-1 to C5 of the substrate to form the product (2o3hex).

We have previously reported RCDM³¹ and parallel path optimizer²⁰ path calculations for the isomerization of 2o4hex by 4OT. We demonstrated that the paths calculated with the parallel path optimizer method agree with the RCDM paths. Furthermore, in the case of the second step, the energy calculated with the parallel path optimizer method is lower than the corresponding RCDM one.

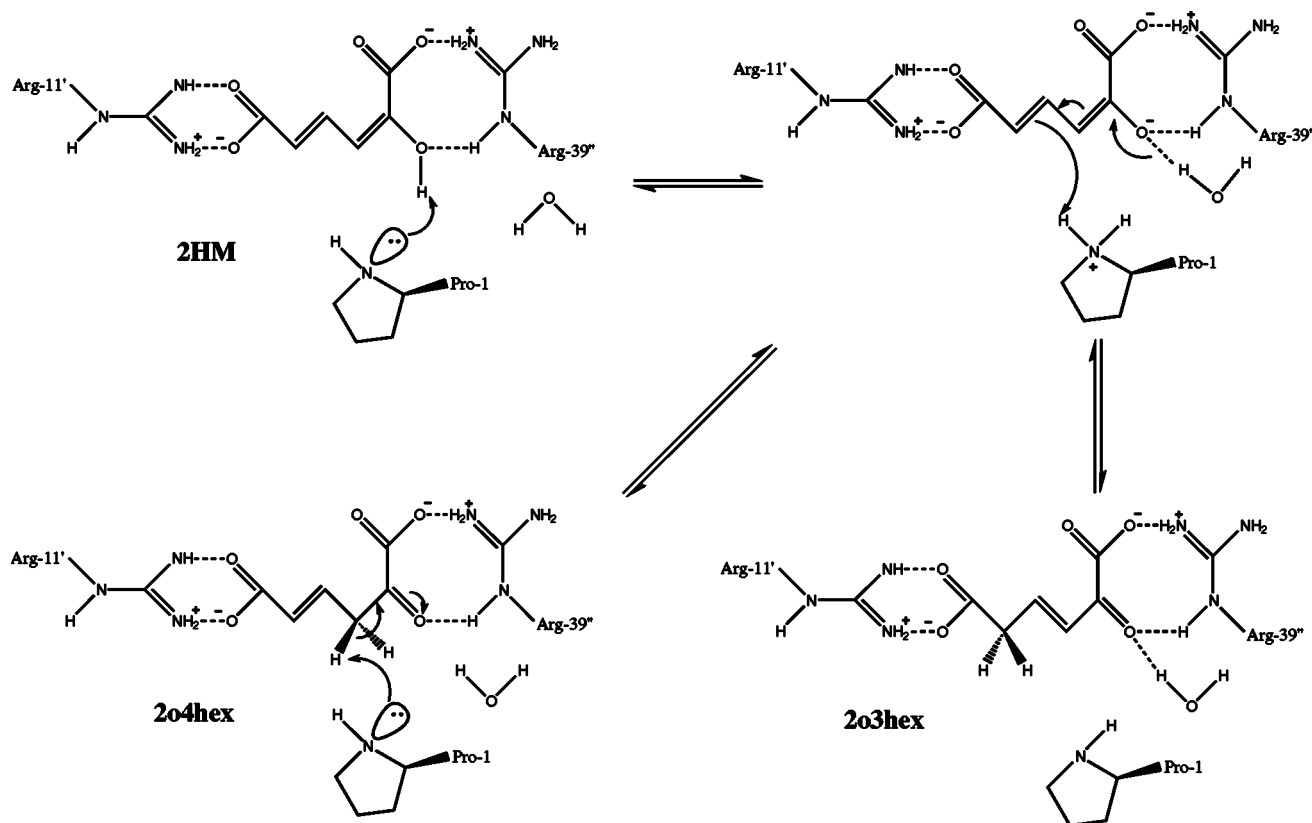


FIG. 1. Suggested mechanism.

In this work we have used the combined procedure as described in Sec. II E to calculate the reaction paths of the two steps of the isomerization of 2o4hex by 4OT. In order to compare the present results with our previous ones, we have chosen to use the reactant, intermediate and product structures from the converged paths from our previous parallel path optimizer method results.²⁰ In all cases the core (QM) subsystem consists of Pro-1, the substrate molecule and a water molecule (36 atoms). The remainder of the enzyme and water sphere are located in the environment (MM) subsystem for a total of 13 197 atoms. An active sphere of 20 Å around C3 of the substrate was used for all optimizations, while the rest of the atoms are kept at fixed positions.³¹ Energies and gradients for NEB and the parallel path optimizer method were calculated at the Hartree-Fock (HF)/3-21G level of theory.

The initial guess for the path in the NEB calculation was obtained by performing a linear interpolation between the end points for each step. For the first step the end points were set to be the reactant and intermediate structures. For the second step the intermediate and product structures were chosen as end points. In both cases we used seven points to simulate each step as in our previous calculations.²⁰ Since NEB is able to calculate reactions with multiple steps, we determined both steps of the reaction concurrently for this part of the procedure. This produced an overall path with 13 points including end points (note that only one image for the intermediate is needed at this point). The initial MM environment chosen for all the points along the path was that of

the product structure. The path was iteratively optimized using restrained MM optimization (see Sec. II C) until convergence.

After the NEB path was converged, it was separated into two steps for the parallel path optimizer method refinement. Each step of the reaction consisted of seven points including the end points. In this case the intermediate point is both the product structure for the first step path and the reactant structure for the second step path. The Cartesian coordinates of the pseudobond atom (the α carbon from Ile-2) and two oxygens from the carboxylate moieties of the substrate were added to the redundant internal coordinates to avoid the rigid body optimization problems as explained in the Methods section.

For the second step path we performed two path optimizer method calculations. In the first calculation (method 1), all seven points in the path were optimized including the intermediate. In the second one (method 2), the minimized structure of the intermediate from the optimized first step path was employed for the path optimization. Thus, only six points were optimized in this last optimization.

Figure 2 shows the comparison of our previous parallel path optimizer method results with the paths determined with the combined method. As can be seen, NEB provides a good initial approximation to the path although it overestimates the energy barrier for both steps of the reaction. It is important to note that in the case of the second step, the NEB path presents two points with almost the same energy which are equidistant from the true TS. This shows NEB's propensity to

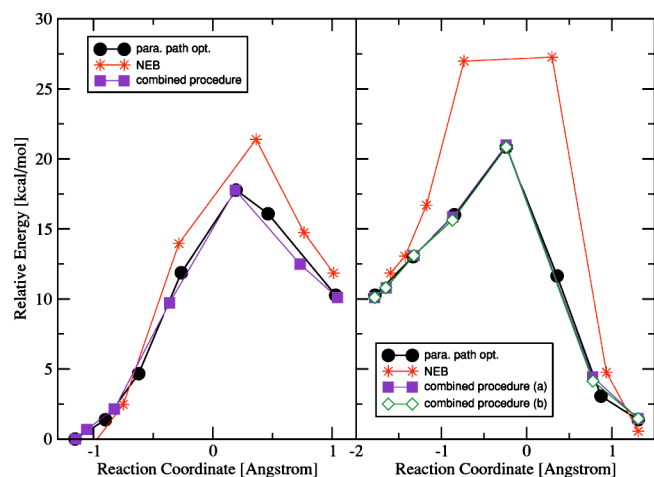


FIG. 2. Reaction path profiles for the first and second steps of the 4OT catalyzed reaction. (a) corresponds to the path optimization with all seven points optimized. (b) corresponds to the path optimization with the minimized intermediate structure from the first step path.

“cut corners” around critical points if there are not enough images to sample the path, or if no special measure is taken to optimize a particular image to the TS.¹⁶

In the case of the paths calculated with the parallel path optimizer method and the combined procedure, all path procedures show very good agreement. The only significant difference between the paths calculated with the parallel path optimizer method only and the combined procedure is the distribution of the images along the path. This difference results from the fact that the initial image distribution from the NEB path is different from the image distribution in the original parallel path optimizer method.

Both paths for the second step obtained from the initial NEB path, method 1 and method 2, overlap. This demonstrates that the intermediate structures obtained by using the full seven point path optimization for the first and second steps correspond to the same minimum. Moreover, the rms deviation including all atoms between both intermediates in the paths obtained with method 1 is 6×10^{-3} Å, with an energy difference of 2×10^{-3} kcal/mol, resulting in a smooth connection between the two steps of the reaction.

As can be seen from Table I, we obtained a significant speedup with the combined procedure over the parallel path optimizer method alone. In this example we observed a reduction of 45%–55% in the number of path optimization cycles required to converge the paths, which effectively increases the speed of the path calculations by approximately a factor of 2 with respect to the parallel path optimizer method calculation alone. These results also show that the parallel

TABLE I. Total QM path optimization iterations for both steps of the isomerization of 2o4hex by 4OT.

| Method | First step | Second step |
|-------------------------|------------|------------------------------------|
| para. path optimization | 765 | 618 |
| Combined procedure | 348 | 339, ^a 306 ^b |

^aCorresponds to the path optimization with all seven points optimized.

^bCorresponds to the path optimization with the minimized intermediate structure from the first step path.

path optimizer method achieves better convergence if a better initial path estimate is employed as would be expected.

IV. CONCLUSIONS

We have developed an efficient procedure for the calculation of MEPs for enzyme reactions, which combines a first order and a second order chain-of-replicas method, NEB and the parallel path optimizer method. The path is approximated by a small number of images that are initially optimized with NEB to obtain a rough guess to the path. This rough guess is subsequently refined with the parallel path optimizer method to obtain the final converged MEP. This procedure combines the speed and functionality of the NEB method with the accuracy of the parallel path optimizer method to provide a robust and general method for reaction path calculations.

This methodology was tested by calculating the isomerization of 2o4hex by 4OT. Our results for the calculated paths show very good agreement with previously published results.²⁰ Moreover, we observe a decrease of 45%–55% in the number of path optimization cycles required to obtain a fully converged path by using the combined procedure over the parallel path optimizer method alone.

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