Supporting Methods

Initial Coordinates Preparation. The starting coordinates of TtCM were modified from the x-ray structure of the F55S mutant of the TtCM trimer [Protein Data Bank (PDB) ID code 1ODE, resolution: 1.65 Å] (1). To revert to the WT TtCM, the 55th residues (serine) in each subunit of the 1ODE were changed back to phenylalanine. [The 55th residue, phenylalanine was mutated to serine during the experiments, according to personal communication with T. H. Tahirov, the author of the 1ODE X-ray structure.] The structures of the WT monomer (PDB ID code 1UFY) and the F55S mutant monomer (PDB ID code 1UI9) have similar rms deviation for the Cα atoms in the two structures (0.0017 Å). Thus, the structure of the WT TtCM trimer should be very similar to that of 1ODE. Finally, a chorismate substrate was docked into one of the three active sites of the TtCM by using SYBYL 6.8 (2). Because of the similarity of the TtCM and BsCM, the x-ray structure of BsCM with the TS analog (PDB ID code 2CHT) was used as a reference structure during the docking of chorismate into the TtCM. The chorismate-bound TtCM structure was energy-minimized by using the CHARMM27 force field after docking.

QM/MM Simulations Setup Procedure. Chorismate was treated as the QM region. Stochastic boundary conditions were applied to the system (3). The residues beyond a distance (r) of 25 Å from the center of the QM region were not included in the calculations. Regions of $r < 23$ Å were chosen as the reaction zone, regions of 23 Å < r < 25 Å were chosen as the buffer zone, and regions of $r > 25$ Å were chosen as the reservoir zone. The atoms in the reaction zone, excluding that in the QM region, were treated with Newtonian dynamics. The atoms in the buffer zone were restrained by harmonic constraints and simulated by using Langevin dynamics. The friction constants used in the integration of the Langevin equation were 250 and 62 ps^{-1} for the protein atoms and water molecules, respectively. A spherical boundary potential centered at the origin of the water cap was used to maintain the correct average distribution of water molecules. The SHAKE algorithm (4) was applied to fix the covalent bonds involving hydrogen. A 1-fs time step was used for integration. Energy minimizations were

performed to remove the bad contacts by using the steepest descent and adopted basis Newton-Raphson algorithms (5), initially with the atoms in the QM region fixed and then with them unfixed. The same procedure was used for the simulations of the chorismate in water. For the reaction in the enzyme, the system was heated from 50 K to the enzyme's optimal growth temperature (343 K) in 50 ps and dynamics were carried out at 343 K. For the reaction in water, the system was heated from 50 K to room temperature (298 K) in 50 ps and dynamics were carried out at 298 K.

Umbrella Sampling and PMF. The starting structures for the umbrella sampling in water and enzyme were extracted from the stable trajectories obtained from the QM/MM dynamic simulations in water and enzyme. For the simulations in the enzyme, the reaction coordinate was divided into 35 windows with 0.1-Å intervals from 1.5 to 5.0 Å. The force constant of the umbrella was 100 kcal•mol^{-1}•Å^{-2}. Four more windows were added around the location of the TS in the reaction coordinate with larger force constants of 200-400 kcal•mol^{-1}•Å^{-2} to improve the degrees of overlap in the phase space between the adjacent windows. Good overlaps in the phase space are essential for obtaining reliable PMF profiles (6). The same procedure was applied for the simulations in water but with 60 umbrella sampling windows and larger force constants because of the rough free energy surface of the reaction in water. A harmonic force constant of 200 kcal•mol^{-1}•Å^{-2} was used for the umbrella sampling windows along the reaction coordinate that were not close to the TS. In the vicinity of the TS, 800-1,000 kcal•mol^{-1}•Å^{-2} force constants were used. For each umbrella sampling window of the enzymatic reaction, the system was energy-minimized by using the steepest descent algorithm, heated from 50 to 343 K in 50 ps, and equilibrated at 343 K for 50 ps. For the simulations in water, the same process was followed but with the equilibration at 298 K. Data were collected at every 100 time steps during the production run for 400 ps after the equilibration. $R_{C5\text{-}C16}$, θ1, and θ2 (Fig. 1*b*) were calculated and analyzed by the weighted histogram analysis method (7, 8). 1D PMF versus $R_{C5\text{-}C16}$ was obtained. To characterize the conformations of the NAC and TS, 1D PMF was projected to 2D PMF in respect to the angles θ1 and θ2 of the reaction coordinates. Single point energies of the TS structures in enzyme and water

were calculated at the SCCDFTB/MM level, and the Mulliken charge distributions for each atom in the QM region were obtained.

Computation of Free Energy for NAC Formation. The free energy of activation is the sum of free energy for NAC formation and the free energy for the conversion of NAC to TS (Eq. **S1**).

$$\Delta G^{\ddagger} = \Delta G^{o}_{NAC} + \Delta G_{TS}. \quad [\mathbf{S1}]$$

By convention, the efficiency of an enzymatic reaction is taken to be the rate constant of the enzymatic reaction divided by the rate constant for the reaction in water at pH 7.0, k_{cat}/k_0 or $[k_{cat}/k_M]\bullet 1/k_0$ (4). In free energy terms, the efficiency is the difference in the free energies of activation ($\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger(w)} - \Delta G^{\ddagger(E)}$) in water and enzyme.

The PMF profiles within the ground-state range were calculated by the weighted histogram analysis method based on the conformations including both NAC and non-NAC. Therefore, the free energy profile in the region of the ground state, represented by 1D PMF, is the combined contributions of the NAC and non-NAC. The free energy for the NAC formation can be calculated from the mole fraction of the ground state present as NAC along the reaction coordinate (ξ) (Eq. **S2**).

$$G_{NAC}(\xi) = G_g(\xi) - RT\ln[P_{NAC}(\xi)] \ . \quad [\mathbf{S2}]$$

In Eq. **S2**, P_{NAC} is the mole fraction of NAC in ground state, R is the gas phase constant, T is the temperature, G_g is the free energy profile of the ground state that is identical to the PMF of the ground state in this article, and G_{NAC} is the free energy profile of NAC formation. The free energy profile of NAC formation is present in the range of $R_{C5\text{-}C16}^{(TS)} < R_{C5\text{-}C16} < 3.7$ Å. $R_{C5\text{-}C16}^{(TS)}$ corresponds to the reaction coordinate where TS is presented. To get an accurate NAC mole fraction (P_{NAC}), umbrella sampling was carried out for 800 ps for each window. The standard free energy for the NAC formation (ΔG_{NAC}^{0}) is calculated from the difference in global minimum of the free energy profile

in the ground state (G_g^{min}) and that of the free energy profile of the NAC formation (G_{NAC}^{min}), as shown in Eq. **S2**.

$$\Delta G_{NAC}^{0} = G_{NAC}^{min}(\xi') - G_{g}^{min}(\xi'') \ . \quad \textbf{[S3]}$$

According to Eq. **S1**, the efficiency of an enzymatic reaction ($\Delta\Delta G^{\ddagger} = \Delta\Delta G_{NAC}^{0} + \Delta\Delta G_{TS}$) can be divided into two parts, which are the differences in the free energies of NAC formation in water and enzyme [$\Delta\Delta G_{NAC}^{0} = \Delta G_{NAC}^{0(W)} - \Delta G_{NAC}^{0(E)}$] and the free energy for conversion of NAC to TS in water and enzyme [$\Delta\Delta G_{TS} = \Delta G_{TS}^{(W)} - \Delta G_{TS}^{(E)}$), respectively. The value of $\Delta\Delta G_{NAC}^{0}$ represents the kinetic advantage of the enzyme in forming NAC and contributes to the efficiency of the enzymatic reaction. Correspondingly, the contribution of the TS stabilization to the efficiency of the enzymatic reaction is denoted $\Delta\Delta G_{TS}$.

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