Another Look at the Mechanisms of Hydride Transfer Enzymes with Quantum and Classical Transition Path Sampling

Michael W. Dzierlenga, Dimitri Antoniou, and Steven D. Schwartz*

Department of Chemistry and Biochemistry, University of Arizona

E-mail: sschwartz@email.arizona.edu

*To whom correspondence should be addressed

Computational Methods

Molecular dynamics and QM/MM calculations were performed using the Chemistry at HARvard Molecular Mechanics (CHARMM)[1,2] program with the CHARMM27 parameter force field.[3] The starting point for the YADH simulations was the crystal structure prepared by Plapp, et al. (PDB ID: 2HCY).[4] The crystallized substrate analog, trifluoroethanol, was converted to benzylate using the CGenFF program version 0.9.6[5,6] to generate parameters from the CGenFF force field version 2b7.[7,8] The crystallized co-enzyme analog, 8-iodo nicotinamide adenine dinucleotide (NAD), was substituted with non-iodinated NAD. The Maestro Protein Preparation Wizard[9] was used to predict the protonation states of residues throughout the protein, and, in particular, Histidine 48 was protonated as in the reactive scheme proposed by Plapp, et al.[10] The protein was solvated in a 130 Å sphere of TIP3P water molecules and 20 potassium atoms were added to neutralize the system.

The system was partitioned into quantum and molecular mechanics regions and the generalized hybrid orbital (GHO) method was used to couple them.[11] The quantum region consisted of one of the active sites, consisting of the benzylate, the nicotinamide ring of the NAD, the zinc and the sidechains of the amino acids which coordinate zinc (2 cysteines in thiolate form and one histidine). The linker atoms were the α-carbons of the amino acids and the NC1$'$ carbon in the NAD. The quantum region was simulated using a modified PM3 method[12,13] which is altered to better model Zinc in biological conditions.[14] Minimization was performed by first constraining the protein and minimizing it gradually reducing constraints, first on the protein sidechains, and then on the protein backbone. The system was then heated from 0 to 300 K over the course of 300 ps, and equilibrated for 300 ps.

Transition Path Sampling (TPS) was performed on both YADH and LDH to generate micro-canonical reactive trajectory ensembles. For the calculation on YADH without CMD, one ensemble was generated containing 101 trajectories. The initial reactive trajectory was generated by using a harmonic potential to compress the donor and acceptor and another harmonic potential on the hydride and acceptor. For the auxiliary calculation on LDH, 20 trajectories were generated in

one micro-canonical ensemble, using as the starting trajectory the final trajectory of one of the microcanonical ensembles generated in the study by Masterson, et al.[15]

For completeness, the system preparation is summarized here. The study began with the crystal structure prepared by Read, et. al. (PDB ID: 1I0Z).[16] To allow for catalysis to occur, the substrate mimic oxamate, which was used for crystallography, was changed to pyruvate, the native substrate, by replacing the nitrogen with a methyl group, and the active site histidine (His193) was protonated. A 130 Å sphere of TIP3P water was used to solvate the protein and potassium ions were used for neutralization. One of the active sites in the enzyme was modeled using the AM1 quantum mechanical potential.[17] The quantum region for QM/MM included the nicotinamide ring on NAD, the pyruvate and the sidechain of His193. The quantum region was linked to the classical region by using the GHO method.[11] The linker atoms were the α-carbon of the histidine and the NC1$'$ carbon of the NAD. Before starting TPS, the system was minimized, heated from 0 to 300 K over the course of 300 ps, then equilibrated for 500 ps. The initial reactive trajectory was generated by adding harmonic constraints to QM/MM dynamics.

The integration of the centroid method is summarized below. Details can be found in an earlier paper by Antoniou, et. al.[18] The motion of the ith quantum particle in the centroid formalism is described by

$$m_i \ddot{\boldsymbol{R}}_i = \langle \boldsymbol{F}_i(\boldsymbol{R}_i, ..., \boldsymbol{R}_N) \rangle_c, \tag{1}$$

where m_i is the mass of the particle, $\ddot{\boldsymbol{R}}_i$ is its centroid position, and the c denotes a path integral average. The centroid position is the center of mass of a polymer chain of B beads,

$$\boldsymbol{R}_i^c = \frac{1}{B} \sum_{\beta=1}^{B} \boldsymbol{r}_i^\beta, \tag{2}$$

where particles are referred to by Latin subscripts and beads are referred to by Greek subscripts. Each bead has mass m_i/B and the sum of forces on all the beads is the centroid force $\boldsymbol{F}_i$,

$$\boldsymbol{F}_i(\boldsymbol{R}_1^c, ..., \boldsymbol{R}_N^c) = -\frac{1}{B} \sum_{\beta=1}^{B} \frac{\partial V(\boldsymbol{r}_i^\beta)}{\partial \boldsymbol{r}_i^\beta}. \tag{3}$$

In Eq. (1) the path integral average is weighted by $\exp(-\beta V_{\text{eff}})$, where V_{eff} is

$$V_{\text{eff}}(\boldsymbol{r}_i^\beta) = \sum_i \sum_\beta \left[\frac{1}{2} k_i (\boldsymbol{r}_i^\beta - \boldsymbol{r}_i^{\beta+1})^2 + \frac{1}{B} V(r_i^\beta) \right], \tag{4}$$

with neighboring beads attached by harmonic potentials with a spring constant of $k_i = m_i B (k_{\text{B}} T)^2$. To efficiently calculate the average of the forces in Eq. (1), a normal mode transformation is applied to separate the slower centroid motion from the motions of the beads.[19] The transformed effective bead potential is

$$V_{\text{eff}}(\boldsymbol{R}_i; \boldsymbol{q}_\alpha^i) = \sum_{i=1}^{N} \sum_{\beta=i}^{B-1} \left[\frac{1}{2} m_\alpha^i (\omega_\alpha^i \boldsymbol{q}_\alpha^i)^2 + \frac{1}{B} V(\boldsymbol{R}_i; \boldsymbol{q}_\alpha^i) \right], \tag{5}$$

where the Bth mode corresponds to the centroid motion. The masses of the normal modes are taken to equal $m_\alpha^i = \mu_i (\omega_\alpha^i)^2$, where μ_i is a proportionality constant.

The normal mode transformation allows for several approximations to be made.[20] First, the normal modes are taken to be fast compared to the centroid motion, which requires a multi-step molecular dynamics propagation.[21] Second, Nosé-Hoover thermostats were attached to each normal mode to properly sample the accessible phase space. Third, since the phase space of the normal mode coordinates is being sampled quickly, the path integral average of the force can be replaced with the instantaneous value of the force for propagation of the centroid variable. This is an analogous approximation to the Car-Parrinello method of molecular dynamics.[22] The CMD method was integrated into CHARMM by altering the source code directly. During the energy calculation of each timestep, the centroid code is activated which calculates the force on the centroid particle over many smaller bead propagation timesteps. This force then replaces the original force on the particle and CHARMM dynamics continues.

For the centroid calculations, each particle was composed of a necklace of 8 beads and the normal modes were propagated with a time-step of 0.05 fs, with a full MD time-step of 0.5 fs. These parameters were chosen as a compromise between accuracy and practicality. Additional beads in the centroid calculation were prohibitively time consuming, while a slower timestep was neces-

sary for the semi-empircal QM method to properly converge. In YADH, the CMD1 and CMD-D ensembles contained 35 reactive trajectories each. For the CMD3 ensemble, 20 trajectories were generated. In LDH 14 reactive trajectories were generated using CMD.

The work calculation performed to find the barrier to hydride transfer is shown below

$$W = -\int F \cdot ds \tag{6}$$

where W is the work on the hydride, F is the force on the hydride, and ds is the path of the hydride projected onto the D–A axis as shown below.

$$ds = -\vec{r}_{\mathrm{H}} \cdot \frac{\vec{r}_{\mathrm{DA}}}{|\vec{r}_{\mathrm{DA}}|} \tag{7}$$

This projection removes the non-reactive motion of the hydride from the work calculation, which simplifies analysis. The distances were calculated in the reference frame of the donor carbon. The work barrier for transfer was averaged across the ensemble to achieve the activation free energy for the hydride transfer.

References

(1) Brooks, B.; Bruccoleri, R.; Olafson, B.; States, D.; Swaminathan, S.; Karplus, M. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **1983**, *4*, 187–217.

(2) Brooks, B. R.; Brooks, C. L.; Mackerell, A. D.; Nilsson, L.; Petrella, R. J.; Roux, B.; Won, Y.; Archontis, G.; Bartels, C.; Boresch, S. et al. CHARMM: the biomolecular simulation program. *J. Comp. Chem.* **2009**, *30*, 1545–1614.

(3) Field, M.; Bash, P.; Karplus, M. A combined quantum mechanical and molecular mechanical potential for molecular dynamics simulations. *J. Comput. Chem.* **1990**, *11*, 700–733.

(4) Plapp, B. V.; Savarimuthu, B. R.; Ramaswamy, S. Protein Data Bank, entry 2HCY.

(5) Vanommeslaeghe, K.; MacKerell Jr., A. D. Automation of the CHARMM general force field (CGenFF)

I: bond perception and atom typing. *J. Chem. Inf. Model.* **2012**, *52*, 3144–3154.

(6) Vanommeslaeghe, K.; Raman, E. P.; MacKerell Jr., A. D. Automation of the CHARMM general force field (CGenFF) II: assignment of bonded parameters and partial atomic charges. *J. Chem. Inf. Model.* **2012**, *52*, 3155–3168.

(7) Vanommeslaeghe, K.; Hatcher, E.; Acharya, C.; Kundu, S.; Zhong, S.; Shim, J.; Daria, E.; Guvench, O.; Lopes, P.; Vorobyov, I. et al. CHARMM general force field: a force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J. Comput. Chem.* **2010**, *31*, 671–690.

(8) Yu, W.; He, X.; Vanommeslaeghe, K.; MacKerell Jr., A. D. Extension of the CHARMM general force field to sulfonyl-containing compounds and its utility in biomolecular simulations. *J. Comput. Chem.* **2012**, *33*, 2451–2468.

(9) Schrödinger Suite 2012 Protein Preparation Wizard, Schrödinger, LLC, New York, NY, 2012: Epik version 2.3, Impact version 5.8, Prime version 3.1.

(10) Plapp, B. V.; Ganzhorn, A.; Gould, R.; Green, D.; Jacobi, T.; Warth, E.; Kratzer, D. In *Enzymology and molecular biology of carbonyl metabolism*, 3rd ed.; Weiner, H., Wermuth, B., Crabb, D., Eds.; Plenum Press: NewYork, 1990; Chapter Catalysis by yeast alcohol dehydrogenase, pp 241–251.

(11) Gao, J.; Amara, P.; Alhambra, C.; Field, M. A generalized hybrid orbital (GHO) approach for the treatment of link-atoms using combined QM/MM potentials. *J. Phys. Chem. A* **1998**, *102*, 4714–4721.

(12) Stewart, J. Optimization of parameters for semiempirical methods I. Method. *J. Comput. Chem.* **1989**, *10*, 209–220.

(13) Stewart, J. Optimization of parameters for semiempirical methods II. Applications. *J. Comput. Chem.* **1989**, *10*, 221–264.

(14) Brothers, E. N.; Suarez, D.; Deerfield II, D. W.; Merz Jr., K. M. PM3-compatible zinc parameters optimized for metalloenzyme active sites. *J. Comput. Chem.* **2004**, *25*, 1677–1692.

(15) Masterson, J. E.; Schwartz, S. D. Changes in protein architecture and subpicosecond protein dynamics impact the REaction catalyzed by lactate dehydrogenase. *J. Phys. Chem. A* **2013**, *117*, 7107–7113.

(16) Read, J.; Winter, V.; Eszes, C.; Sessions, R.; Brady, R. Structural basis for altered activity of M- and H-isozyme forms of human lactate dehydrogenase. *Proteins: Struct., Funct., Genet.* **2001**, *43*, 175–185.

(17) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. A.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

(18) Antoniou, D.; Schwartz, S. D. Approximate inclusion of quantum effects in transition path sampling. *J. Chem. Phys.* **2009**, *132*, 224111.

(19) Cao, J.; Voth, G. The formulation of quantum statistical mechanics based on the Feynman path centroid density. IV. algorithms for centroid molecular dynamics. *J. Chem. Phys.* **1994**, *101*, 6168–6183.

(20) Kinugawa, K.; More, P.; Klein, M. Centroid path integral molecular dynamics simulation of lithium para-hydrogen clusters. *J. Chem. Phys.* **1997**, *106*, 1154–1169.

(21) Martyna, G.; Tuckerman, M.; Tobias, D.; Klein, M. Explicit reversible integrators for extended dynamics. *Molecular Physics* **1996**, *87*, 1117–1157.

(22) Car, R.; Parrinello, M. Unified approach for molecular-dynamics and density-functional theory. *Phys. Rev. Lett.* **1985**, *55*, 2471–2474.