A QM/MM Free Energy Study of the Oxidation Mechanism of Dihydroorotate Dehydrogenase (Class 1A) from *Lactococcus lactis*

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Supporting Information

1. Correction of SCC-DFTB/MM by B3LYP/MM method

To correct the possible inaccuracies in the SCC-DFTB method and verify its reliability with a higher Hamiltonian, we carried out single-point B3LYP/MM calculations using representative snapshots from MC and TS states, which are the same used on the Interaction Energy Decomposition analysis. The corrected SCC-DFTB/MM results based on the B3LYP/MM calculations improve not only the QM level as well as the MM interactions and its polarization on the QM subsystem[1]. The SCC-DFTB correction was performed using a straightforward one-step free energy perturbation,

$$\Delta G_{\mathrm{B3LYP-DFTB}} = -k\mathrm{T}\ln\langle e^{-\beta(\mathrm{E_{B3LYP/MM}}-\mathrm{E_{DFTB/MM}})}\rangle_{\mathrm{DFTB/MM}}$$

which was done at both MC and TS states. The difference between the perturbative correction at these two states gives the B3LYP/MM correction to the free energy of the half-reaction catalyzed by *L. lactis* DHOD. The B3LYP/6-31G* was applied in order to perform the correction. The free energy barrier obtained by SCC-DFTB/MM method is estimated at 5.64 kcal/mol, whereas the free energy barrier calculated by B3LYP/MM is 5.06 kcal/mol. Therefore, the free energy difference between SCC-DFTB and B3LYP method is just 0.58 kcal/mol, showing the high reliability of the semiempirical method (SCC-DFTB) to the high Hamiltonian (B3LYP).

1. Riccardi, D.; Schaefer, P.; Cui, Q. Pk(a) Calculations In Solution And Proteins With QM/MM Free Energy Perturbation Simulations: A Quantitative Test Of QM/MM Protocols. *J. Phys. Chem. B* **2005**, *109*, 17715-17733.