Supporting Information for

Insights into Catalytic Mechanism of Unsaturated Glucuronyl Hydrolase of *Bacillus sp.* GL1

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Supporting Information

1. Average distances between the carboxylate group of D88 and HO2/HO3 of ΔGlcA unit.

2. Minimum energy pathway and CPR optimized energetic profile for pathway I using SCC-DFTB/MM approach.

3. Minimum energy pathways and CPR optimized energetic profiles for pathway II using SCC-DFTB/MM approach.

4. Overlay representations of EI from pathway and from pathway .

5. Snapshot of ES with Nδ of H193 unprotonated.

6. Full citation for Ref. 24

Riccardi, D.; Schaefer, P.; Yang, Y.; Yu, H.; Ghosh, N.; Prat-Resina, X.; Konig, P.; Li, G.; Xu, D.; Guo, H.; Elstner, M.; Cui, Q., Development of Effective Quantum Mechanical/Molecular Mechanical (QM/MM) Methods for Complex Biological Processes. J. Phys. Chem. B 2006, 110, 6458-6469.

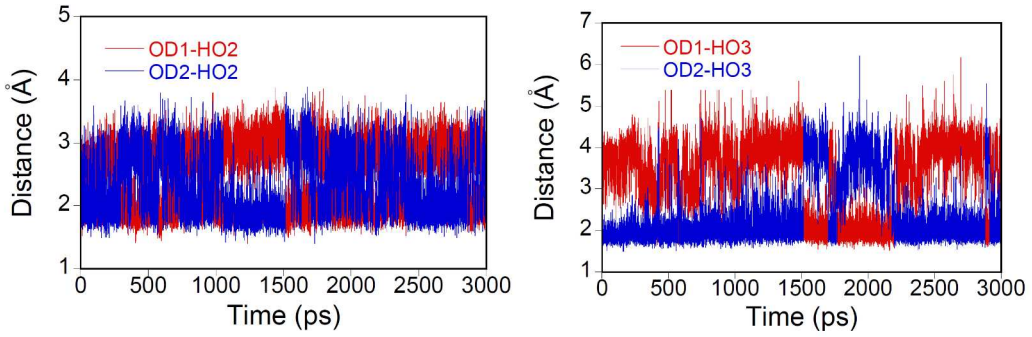
7. Full citation for Ref. 30

MacKerell Jr., A. D.; Bashford, D.; Bellott, M.; Dunbrack Jr., R. L.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.;

Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher III, W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M., All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins. *J. Phys. Chem.* **1998,** B102, 3586-3616.

8. Full citation of Ref. 43

Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J., J. A. ; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, E.; Yazyev, O.; Austin, J.; Cammi, R.; Pomelli, C.; Ochterski, W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski; Fox, J. *Gaussian 09, Revision A.01*, Gaussian, Inc.: Wallingford CT, 2009.



Figures S1. Average distances between the carboxylate group of D88 and HO2 of ΔGlcA (left panel) or HO3 of ΔGlcA (right panel).

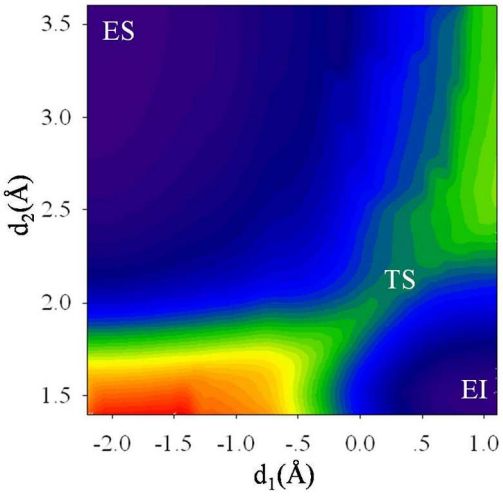


Fig S2. Minimum energy path for the mechanism **I** calculated using the SCC-DFTB/MM method, in which $d_1 = d_{OD2(D149)\cdots HD2} - d_{C4\cdots HD2}$, $d_2 = d_{C5\cdots Ow}$.

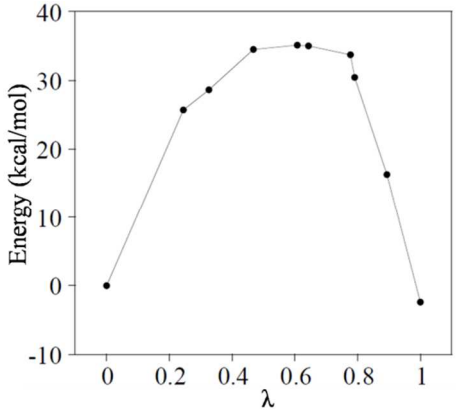


Fig S3**.** CPR-refined energetic profiles using the SCC-DFTB/MM approach for the hydration reaction undergoing mechanism **I**.

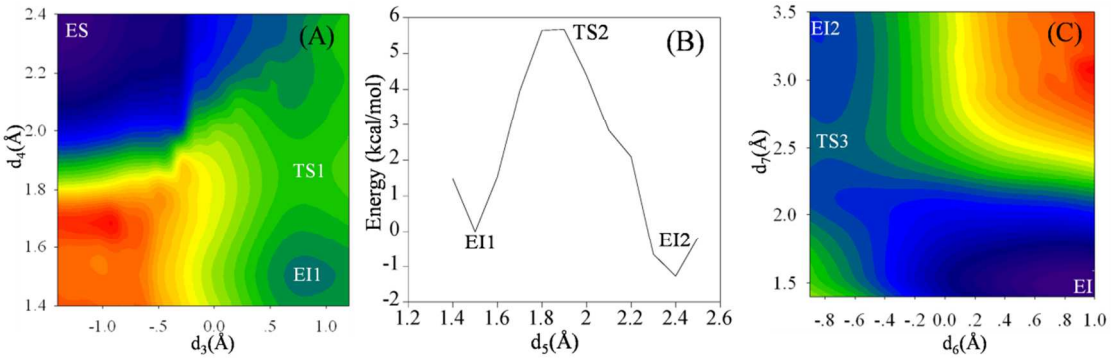


Fig S4**.** Minimum energy path for the mechanism **II** along the putative reaction coordinates calculated using the SCC-DFTB/MM method, in which $d_3 = d_{\mathrm{OD2(D149)\cdots HD2}} - d_{\mathrm{C4\cdots HD2}}$, $d_4 = d_{\mathrm{O2\cdots C1}}$, $d_5 = d_{\mathrm{O2\cdots C1}}$, $d_6 = d_{\mathrm{OD2(D149)\cdots H_2}} - d_{\mathrm{Ow\cdots H_2}}$ and $d_7 = d_{\mathrm{C5\cdots Ow}}$.

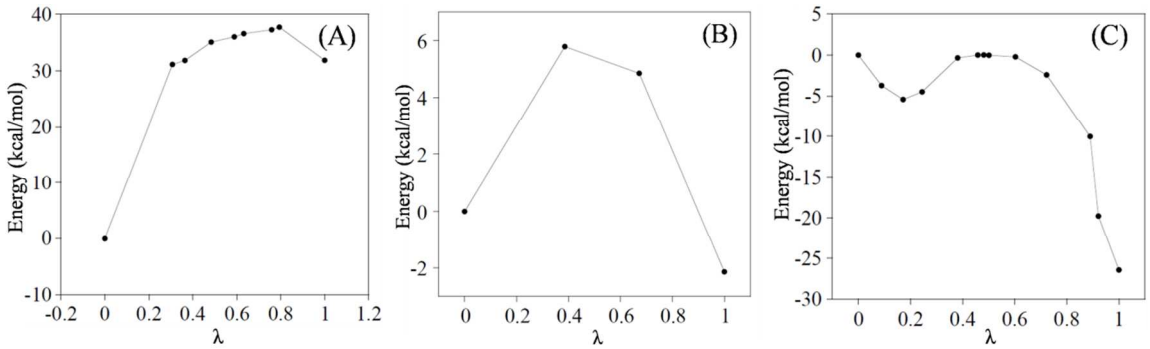


Fig S5**.** CPR-refined energetic profiles using the SCC-DFTB/MM approach for each step in the hydration reaction undergoing mechanism **II**.

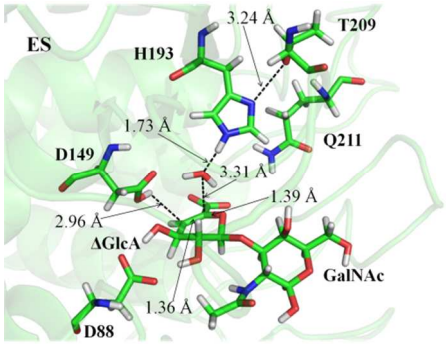


Fig S6. Overlay representations of EI from mechanism (carbon in green) and from mechanism (carbon in magenta).

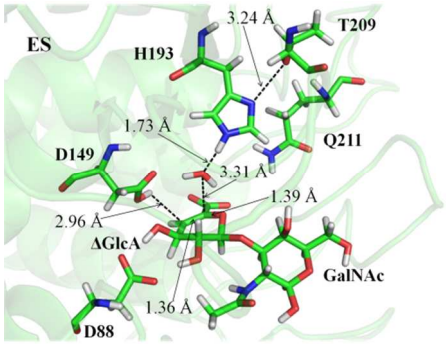


Figure S7. Snapshot of ES with Nδ of H193 unprotonated.