Supporting Information

Insights into Enzymatic Catalytic Mechanism of bCinS: The Importance of Protein Conformational Change

Jingyuan Zhuang,[1,2#] Fan Zhang,[1#] Xiaowen Tang,[3] Chengzhi Liu,[4] Min Huang,[4] Hujun Xie,[4,*] Ruibo Wu[1,*]

[1] *School of Pharmaceutical Sciences, Guangdong Provincial Key Laboratory of New Drug Design and Evaluation, Sun Yat-sen University, Guangzhou 510006, People's Republic of China*

[2] *Warshel Institute for Computational Biology, School of Life and Health Sciences, The Chinese University of Hong Kong, Shenzhen, 518172, People's Republic of China*

[3] *Department of Medicinal Chemistry, School of Pharmacy, Qingdao University Medical College, Qingdao 266073, People's Republic of China*

[4] *School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310018, People's Republic of China*

[#] These authors contributed equally to this work.
[*] To whom correspondence should be addressed:
hujunxie@gmail.com; wurb3@mail.sysu.edu.cn;.

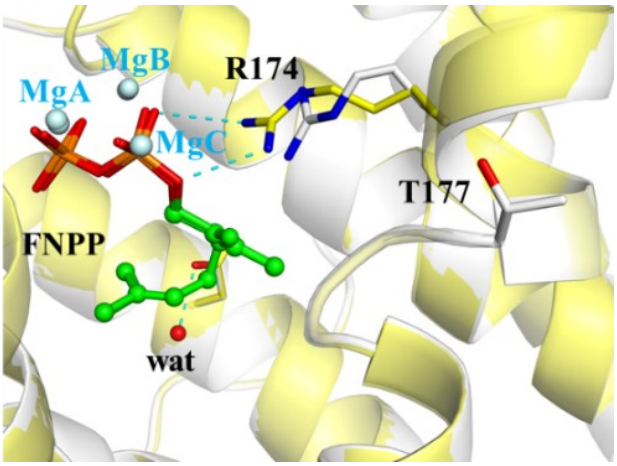

Figure S1. Superpose of the bCinS-FNPP crystal structures with $2Mg^{2+}$ (MgA and MgB, white) and $3Mg^{2+}$ (MgA, MgB and MgC, yellow).

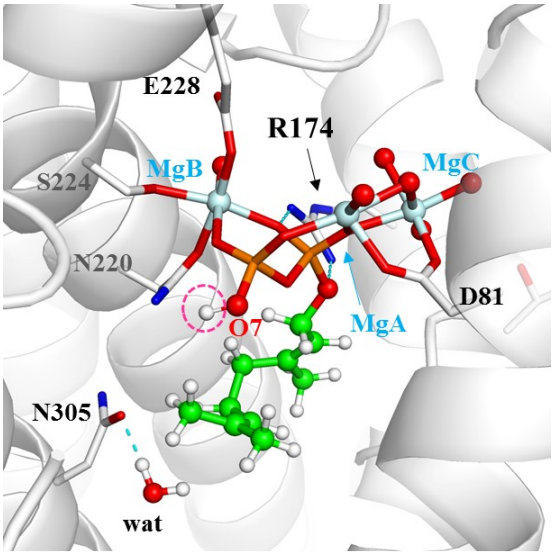

Figure S2. The selection of QM regions and the potential protonation site labeled as O7. The red spheres coordinating with Mg are the water molecules. Mg ions, substrate, wat, N305, R174, D81 and E228 are contained in QM1, while MgA, MgB, substrate, wat and N305 are included in QM2. The hydrogen bond interaction is shown by a cyan dashed line.

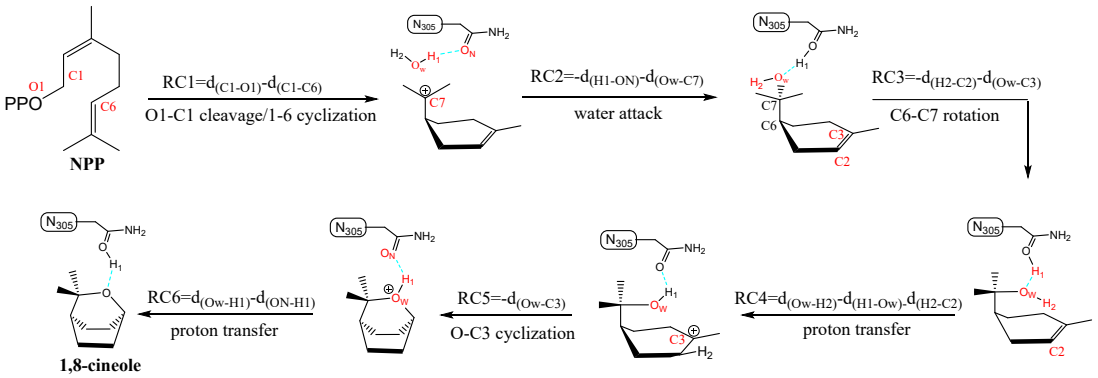

Figure S3. The defined reaction coordinates (RC) for biosynthesis of 1, 8-cineole by bCinS-catalyzed NPP cyclization.

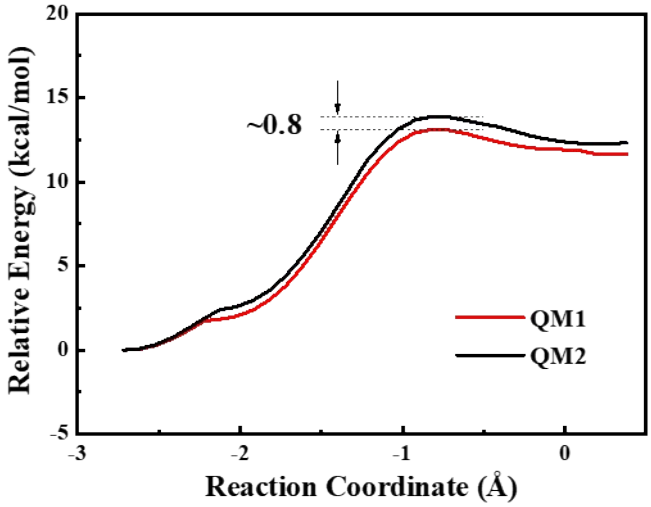

Figure S4. The QM/MM benchmark test of proton transfer in heterocyclization with QM1 and QM2.

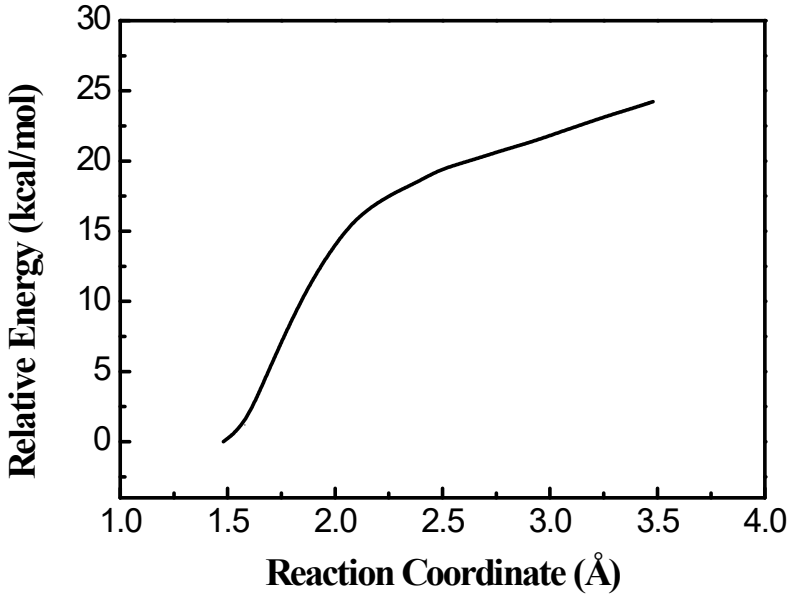

Figure S5. The relative energy profile of the PPi cleavage along the C1-O1 bond. There is no stable or metastable state intermediate state after the PPi cleavage.

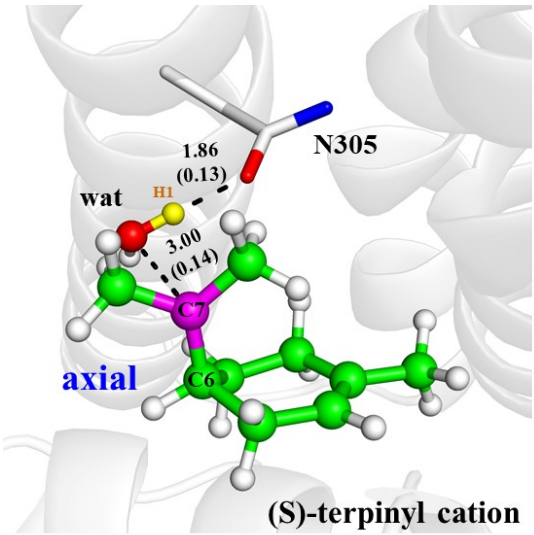

Figure S6. Another view of IM1'. The axial single bond of C6-C7 is preorganized for the subsequent water attack and heterocyclization.

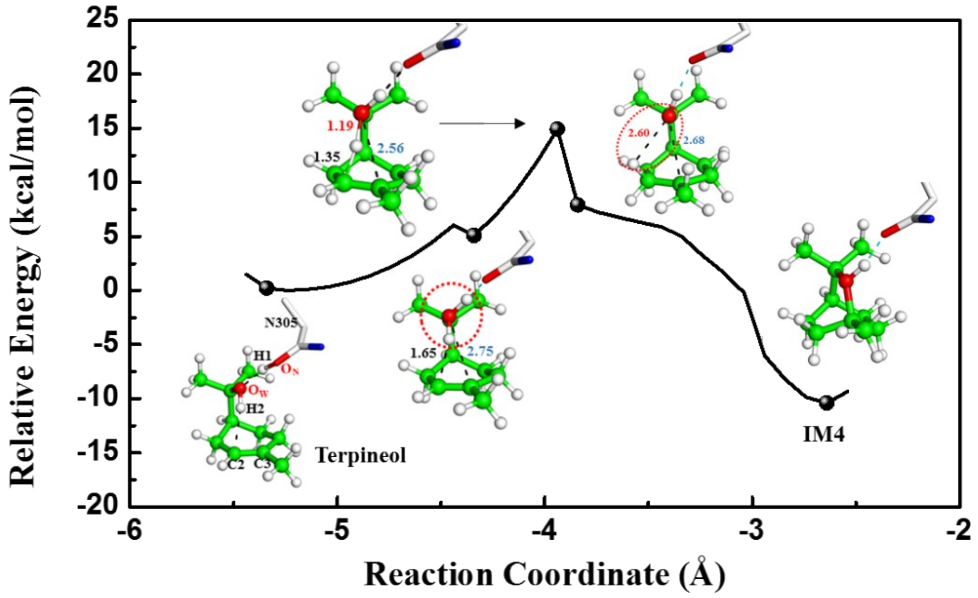

Figure S7. The relative energy profile and representative structures of heterocyclization under the concerted addition mechanism (RC=-d(H2-C2)-d(OW-C3)).

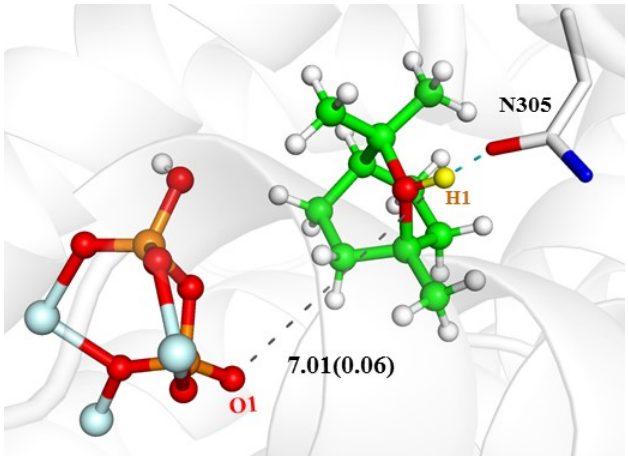

Figure S8. The representative structure of IM4. O1 is too far away from H1 to serve as the direct proton acceptor.

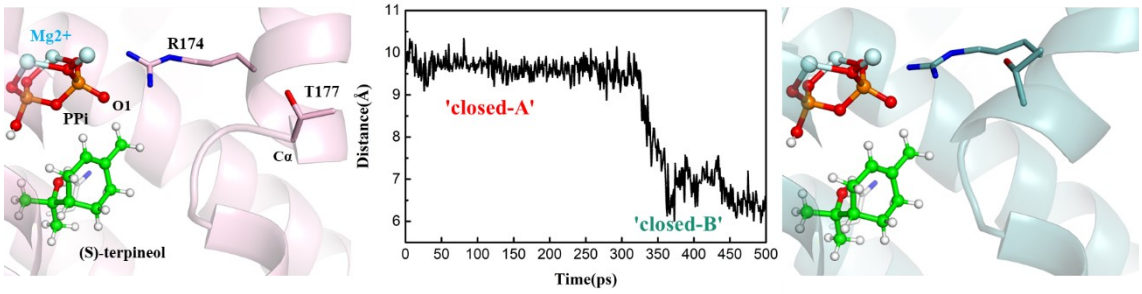

Figure S9. The conformational change in classical MD simulations with the frozen QM subsystem of the sample window between (S)-terpineol and terpinyl hydronium ion. The distance between O1 of PPi and Cα of T177 used to detect the state evolution decreases from nearly 10Å to about 7Å. The structure with the pink cartoon is in 'closed-A' state and the teal one is in 'closed-B' state.

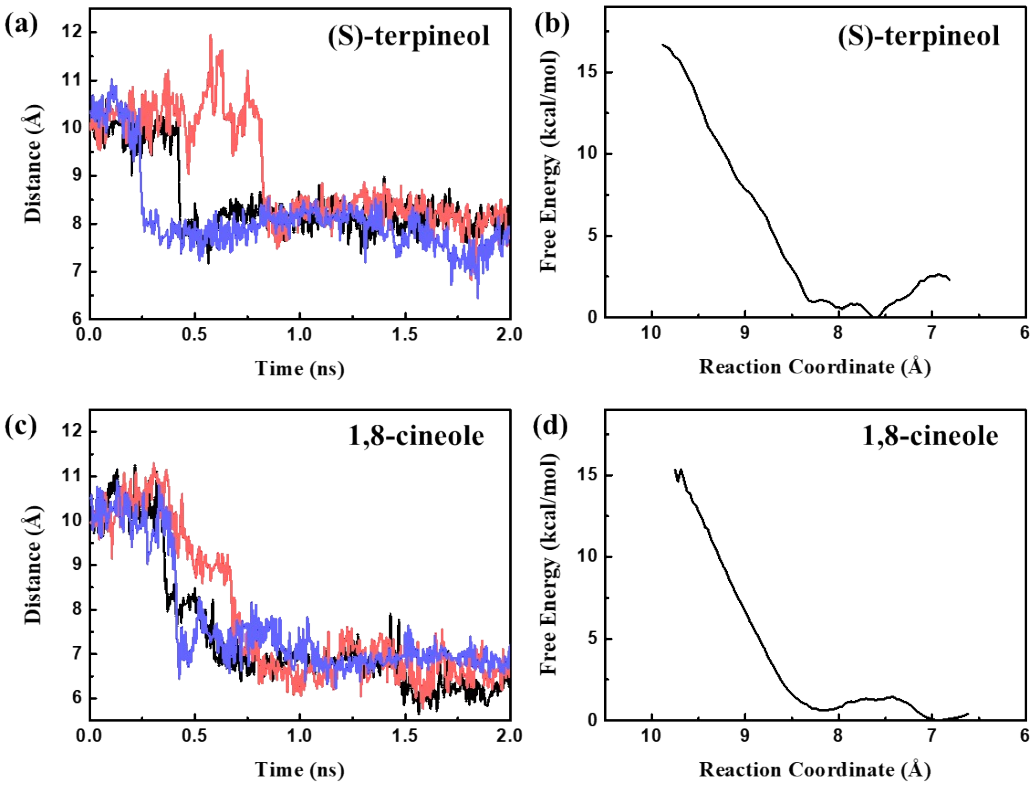

Figure S10. The change of distance between O1 of PPi and Cα of T177 (a, c) and the free energy profiles of the kink region movement (b, d). The distance reducing from nearly 10Å to about 7Å and the energy release without energy barrier indicate that the converting from 'closed-A' to 'closed-B' state is spontaneous in bCinS.

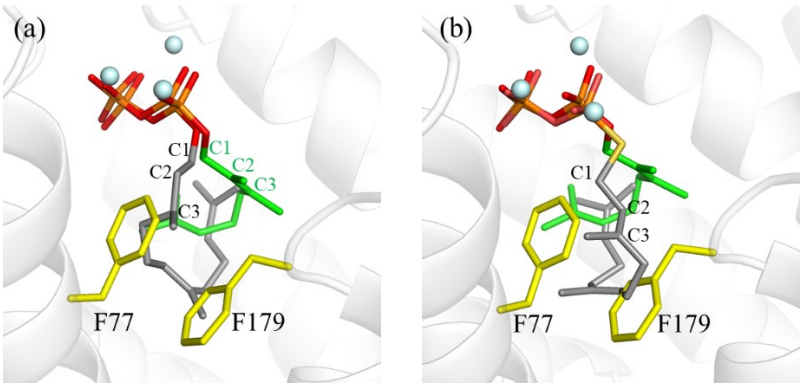

Figure S11. Superimposition of the substrate analogues in SdS (a) and AtAS (b) crystal structures to the active site pocket of bCinS. The carbon chain of C15 substrate is rendered in gray and C10 substrate in green.