Supporting Information:

Switching on a Nontraditional Enzymatic Base – Deprotonation by Serine in the *ent*-Kaurene Synthase from *Bradyrhizobium japonicum*

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1. Computational Study

1.1 DFT calculations

1.1.1 Relative energies of three possible deprotonation product

Table S1 Relative energies (in kcal/mol) of three possible deprotonation products in different level of theory and solvent.

Level of theory	Solvent	2	3	4
ωB97XD/6-311+G(d,p)	PCM/Water	0.54	0.73	0
ωB97XD/6-311+G(d,p)	PCM/Chloroform	0.32	0.9	0
mPW1PW91/6-311+G(d,p)	PCM/Chloroform	2.07	2.13	0
BB1K/6-311+G(d,p)	PCM/Chloroform	0.92	0.89	0

1.1.2 DFT calculation of optimal angles for proton transfer of model system

Table S2 Energy of DFT 2D scan with wB97XD/6-31+g(d,p) level of theory.

Angle B	155	155	155	155	155	155	155
Angle A	90	100	110	120	130	140	150
Energy(kcal/mol)	-220766.33	-220769.58	-220771.73	-220771.48	-220770.62	-220768.49	-220766.98
Angle B	165	165	165	165	165	165	165
Angle A	90	100	110	120	130	140	150
Energy(kcal/mol)	-220767.54	-220771.34	-220772.9	-220773.07	-220772.39	-220770.85	-220769.04
Angle B	175	175	175	175	175	175	175
Angle A	90	100	110	120	130	140	150
Energy(kcal/mol)	-220768.44	-220771.94	-220773.26	NA	-220772.76	-220771.45	-220769.54
Angle B	185	185	185	185	185	185	185
Angle A	90	100	110	120	130	140	150
Energy(kcal/mol)	-220768.39	-220771.94	-220773.26	NA	-220772.84	-220771.49	-220769.52
Angle B	195	195	195	195	195	195	195
Angle A	90	100	110	120	130	140	150
Energy(kcal/mol)	-220767.54	-220771.34	-220772.9	-220773.05	-220772.38	-220771.24	-220769.16
Angle B	205	205	205	205	205	205	205
Angle A	90	100	110	120	130	140	150
Energy(kcal/mol)	-220766.2	-220769.61	-220771.08	-220771.54	-220771.31	-220770.05	-220768.3
Angle B	215	215	215	215	215	215	215
Angle A	90	100	110	120	130	140	150
Energy(kcal/mol)	-220764.61	-220768.03	-220769.64	-220769.96	-220769.39	-220768.25	-220766.08

1.1.3 Conformational search and energies of **A**

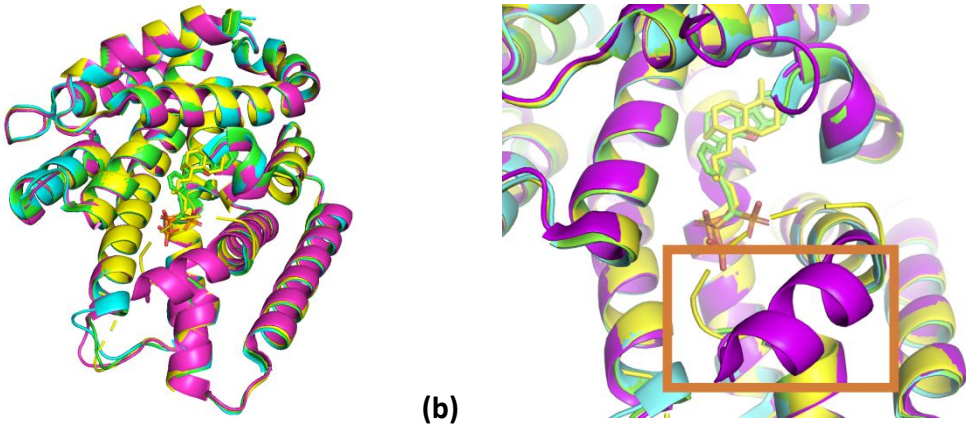
Energy of each conformer is shown in Table S3, only the conformers that are within 5kcal/mol of the lowest energy conformer were kept for docking. All structures are available as mol2 file.

Table S3 Free energies and relative energies of each conformer.

Conformers	Free Energy(kcal/mol)	Relative Energy(kcal/mol)
1	-490173.9459	0
2	-490173.8185	0.12738453
3	-490173.7602	0.18574296
4	-490173.7369	0.20896083
5	-490173.7281	0.21774597
6	-490173.5135	0.43235439
7	-490171.6781	2.26782114
8	-490171.6291	2.31676692
9	-490171.2238	2.72213838
10	-490171.038	2.90788134
11	-490170.8071	3.13880502
12	-490170.6653	3.28062228
13	-490170.2875	3.6583833

1.2 Docking Simulation

1.2.1 Comparison of four crystal structures.



(a) **(b)**

Figure S1 (a) Comparison of four crystal structures of BjKS. 4W4R: apo, cyan. 4W4S: with B29, green. 4XLX: apo, magenta. 4XLY: with CPP, yellow. **(b)** Comparison of the active site of structures in (a). The orange box indicates the region that is complete in 4XLX but is incomplete in the others.

1.2.2 Docking constraint Setup

During the docking simulation, chemically meaningful constraints were applied during the sampling. Specifically, three types of constraints were applied.

1. Chemistry constraints

During the first step of the mechanism, diphosphate-Mg^{2+} complex leaves carbon 14 to form first carbocation intermediate. C14 can leave from two possible oxygens. Since there's no experimental result confirm which one it is, here we apply constraints to examine both possibilities. Shown in Figure S2.

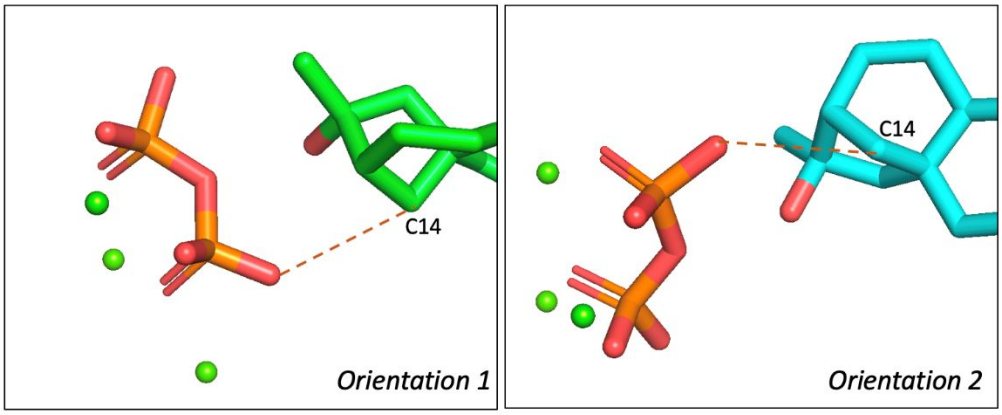


Figure S2 Two possible orientations of the DPP complex leaving in the first step of the reaction mechanism.

2. Coordination constraints

Diphosphate-Mg^{2+} complex is coordinating with the aspartate rich motifs. This set of constraints define the distance/angle/dihedrals to be similar with orientations observed in crystal structures with the diphosphate and all three magnesiums, shown in Figure S3. All constraine values are summarized in Table S4. Two magnesiums on the left were constraint to the DDXXD motif while the magnesium on the right was constraint to N205. The typical NSE motif is NGD in BjKS. MG1 is not constraint to the last aspartate because the absence of the diphosphate-Mg^{2+} complex in the crystal structure might lead to the H helix slightly shift away from the active site. During the docking simulation we allow active site backbone movement and extensive side chain conformation sampling to maximumly overcome this issue.

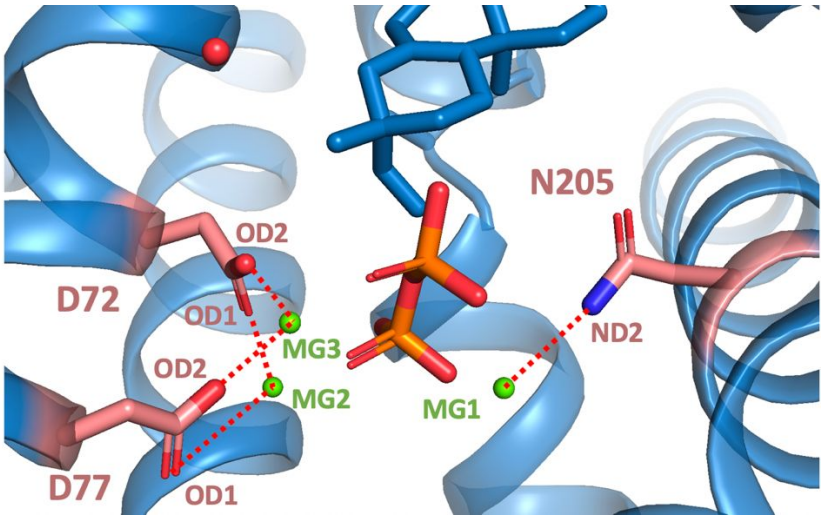


Figure S3 Coordination constraints.

All constraint values were obtained from crystal structures. Weight for distance is 10 times of angle or dihedral angles since distance range is less likely to be outside of the set range. All numbering is from the mol2 file numbering. For water constraint, weight for distance is set to 50, angle B and torsion B is set to 30.

3. Water constraints

Initial docking results using these constraints indicated that E28 and F72 sidechains frequently adopted conformations different from those observed in the crystal structure (Figure S4). It does not seem likely that a nonpolar group would vacate space in the carbocation (hydrocarbon) binding site to be replaced by a polar group. Close examination of structures from docking indicated that the problem might be that a water molecule (Figure S4 left, red) that is present in the crystal structure was absent in the docking simulation (removal of water molecules is a common step in docking procedures). *pyWater* analysis confirmed the conservation of this water molecule in related crystal structures. (Figure S4, right.) The only water that shows conservation in active site is the highlighted large sphere shown in Figure S4, right. Thus, to ensure a reasonable conformation of the important residues in the active site, this water molecule was constrained to the D76 and E28 residues during subsequent docking.

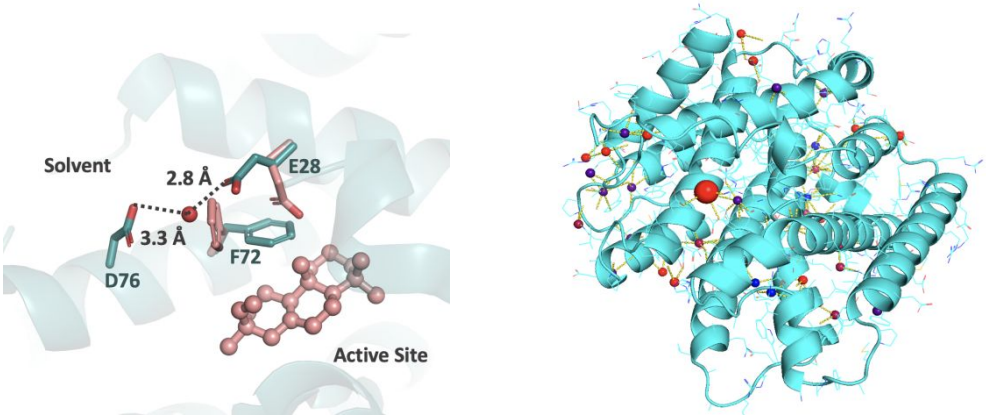


Figure S4. Left: Preliminary docking results with angle constraints. Green: crystal structure. Salmon: docking result for A167S mutant. Docked carbocation **A** shown as a ball-and-stick model. Right: Pywater analysis: All water molecule in the crystal structure were examined and water with conservation over 0.7 were shown in the figure. Darker color suggests higher conservation. The conserved water used in docking is highlighted (red large sphere).

The constraints are set between E26 and D74 with water shown in Figure S5 below. Values are shown in table S4. These values are obtained from crystal structures.

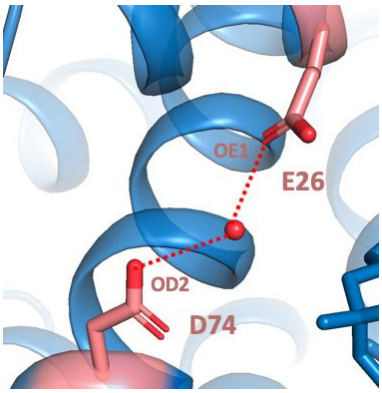


Figure S5 Water constraints.

4. Angle constraints on Ser167

Angle constraints are applied as QM result suggests. Angle A are constrained at 120 degree with 10% deviation while angle B are constrained at 180 with 10% deviation. All the angles are applied to 3 different carbons (5 different proton positions since C7 and C14 has 2 protons attach to them while C9 has one).

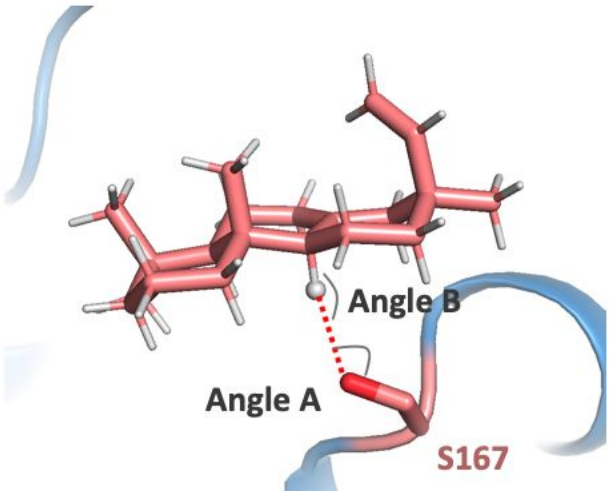


Figure S6 Angle constraints between S167 and intermediate.

Table S4 Docking constraint values for A167S structure.

Chemistry Constraint	Distance		
DPP - C14	3.0 ± 0.5		
Coordination Constraint	Distance	Angle	Torsion
D73_OD2 - MG3	2.5 ± 0.3	145.3 ± 20	128.7 ± 20
D73_OD1 - MG2	2.5 ± 0.5	129.3 ± 20	290.3 ± 20
D77_OD2 - MG3	2.5 ± 0.3	107.2 ± 20	155.1 ± 20
D77_OD2 - MG2	2.5 ± 0.3	129.4 ± 20	220.6 ± 20
N205_ND2 - MG1	2.5 ± 0.5	147.3 ± 20	95.4 ± 20
Water Constraint	Distance	Angle	Torsion
26E_OE1 - Water O	2.8 ± 0.5	103.8 ± 10	191.8 ± 19
74D_OD2 - Water O	3.3 ± 0.5	100.7 ± 10	230.6 ± 23
Angle constraint	Distance	Angle	Torsion
A167S - angle A	NA	120.0 ± 12	NA
A167S - angle B	NA	180.0 ± 18	NA

1.2.3 Diphosphate orientation

In the docking study, diphosphate-Mg^{2+} complex geometry was taken from the closest homologue. To validate the conformation, we examined all class I terpene synthase crystal structures with complete diphosphate-Mg^{2+} complex bound. Including 2ONG, 1N21, 5IKA, 1JFG, 2OA6, 3KB9 and 4OKZ. As shown in Figure S7, the diphosphate-Mg^{2+} complex has very conserved conformations of the binding motif residues (**D**DXX**D** motif and (**N**,**D**)D(L,I,V)X(**S**,**T**)XXX**E** motif, bold are the residues directly interacting with the Mg^{2+}).

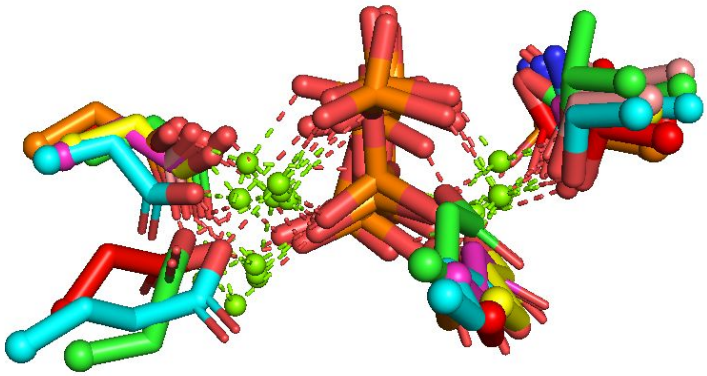


Figure S7 Class I terpene synthase diphosphate-Mg^{2+} conformation overlap with catalytic residues. (Salmon: 2ONG, Green: 1n21, Red: 5ika, Orange: 1jfg, Meganta: 2OA6, Yellow: 3KB9, Cyan: 4OKZ)

1.2.4 Docking result for A167S

The number of poses passed filters are shown in Table S5. C7 and C14 bear two hydrogen (C7: H32, H33, C14: H25, H19), while C9 bears one (H20). Docking runs were carried out individually for each possible deprotonation position and combined for filtering. Result poses for each deprotonated H are available as PDB files, labeled as the proton name. Representative pose shown in Figure 3b is available as m2_c10_h33_a167s.pdb.

For each of the 10 possibilities, 25,000 docking runs were performed, adds up to 250,000 docking runs in total. All docking results were combined and then filtered based on satisfaction of constraints, total protein energy and interface energy. On the first stage of filtering, all docking results are taken into consideration. If a pose satisfies the constraint described in last section with minimal deviation, it'll be kept for further docking. This step left with 82912 poses. Secondly, poses are filtered based on total protein score. Lowest 10% were kept (lower than -738.25 REU, Rosetta energy unit). Thirdly, poses are filtered based on interface energy and lowest 5% were kept (lower than -17.10 REU). That leads to the final poses pass through the filter, summarized

in chart below. All poses are examined and few poses with Ser-H distance greater than 4Å was discarded.

Table S5 Docking results. The darker green a cell is, the more poses passed filter. The sum of poses for deprotonation on each carbon is shown in the last row, with the brown bars representing relative amounts of deprotonation at C7, C14 and C9.

A167S	C7 Orientation 1		C14 Orientation 1		C9 Orientation 1
Deprotonated H	H32	H33	H25	H19	H20
Poses	15	29	38	42	2
	C7 Orientation 2		**C14 Orientation 2**		**C9 Orientation 2**
Deprotonated H	H32	H33	H25	H19	H20
Poses	44	102	7	30	14
SUM	190		117		16

1.2.5 Wild type docking

Four ion-pair orientations were examined individually during the wild-type docking simulation. Carbon 16 and 17 were constraint to either of the two possible oxygens on diphosphate, resulting in four orientations.

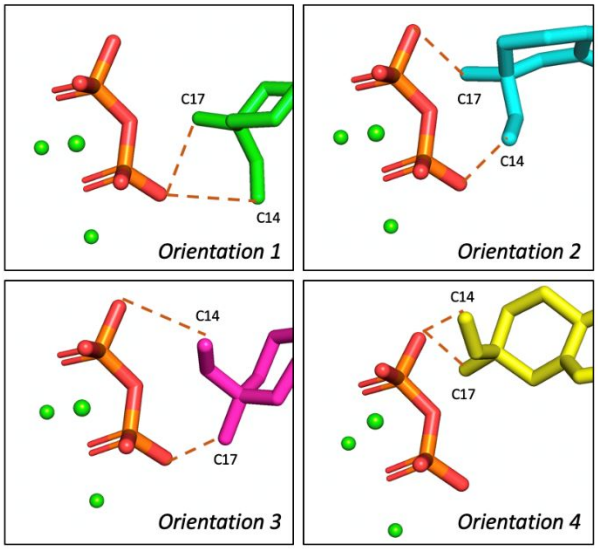


Figure S8 Four possible ion-pair orientations during wild-type docking simulation.

Three hydrocarbon structures: **A**, **C**, and transition state structure A to C **TS(A-C)** were docked in wild-type BjKS. Overlay of representative low energy structure of **A**, **TS(A-C)** and **C** are shown in Figure S9. No significant rotational or translational movement between each structure was observed. Detailed information will be reported in future publication. The result poses are available as PDB files.

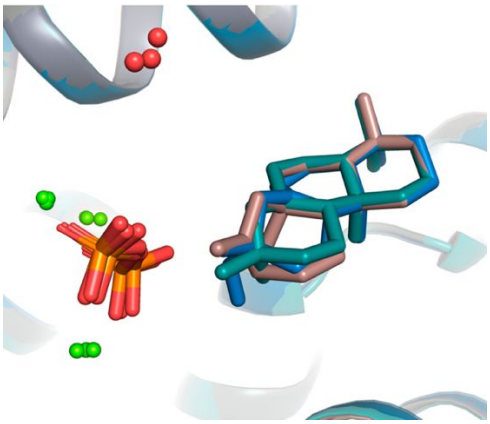


Figure S9 Overlay of **A**(navy), **TS(A-C)** (dark green), **C** (light brown) low energy structures.

Attachment Files

IntmA_Conformers

Optimized conformers of intermediate A.

Rosetta_Input_File

Rosetta input files for docking simulation. Folders are labeled as orientation_carbonName_protonName of each possible orientations and deprotonation position shown in table S5. Each folder contains the following:

4xlx_wlig_1.pdb: the input pdb file for docking.
dock.cst: constraint file.
flags: multiple command line options
ks_dock.xml: xml file for docking procedure
run_dock2.sh: shell script for submitting job
params files and confs.pdb: input conformers and parameter file describing chemical and geometric information for ligands.

Result_Poses

The poses passed filters are attached as PDB files in all_a167s_poses folder. Each subfolder is labeled as the proton name shown in Table S5. The PDB file for Figure 3B is included as m2_c10_h33_a167s.pdb in lowE_models folder. Wild type docking results shown in Figure S9 are attached as PDB files in lowE_models/wildtype.