# Supporting Information

# A highly active and regioselective cannabigerolic acid synthase engineered from a promiscuous prenyltransferase NphB

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**Steady-state concentration of CBGA**

The dependence of the steady-state concentration of CBGA on the rate constant $k_1$ of forming CGBA from OA and GPP was analyzed as below:

$$[OA] + [GPP] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [CBGA] \xrightarrow{k_3} \text{(degradation)}$$

where $k_2$ is the rate of the CBGA decomposition reaction, $k_3$ is the rate of the CBGA degradation, $[OA]_0$ and $[GPP]_0$ are initial concentration of the substrates

Rate of CBGA synthesis = $k_1[OA][GPP]$
Rate of CBGA decomposition = $k_2[CBGA] + k_3[CBGA]$

At the steady-state,
$k_1[OA][GPP] = k_2[CBGA] + k_3[CBGA]$

Replacing [OA] and [GPP] with ($[OA]_0 – [CBGA]$ and $[GPP]_0 – [CBGA]$), respectively
$k_1([OA]_0 – [CBGA])([GPP]_0 – [CBGA]) = k_2[CBGA] + k_3[CBGA]$
$k_1([OA]_0[GPP]_0 – ([OA]_0 + [GPP]_0)[CBGA] + [CBGA]^2) = k_2[CBGA] + k_3[CBGA]$

Dividing both sides with $k_1$,
$[OA]_0[GPP]_0 – ([OA]_0 + [GPP]_0)[CBGA] + [CBGA]^2 = (k_2 + k_3) / k_1[CBGA]$

Dividing both sides with [CBGA],
$[OA]_0[GPP]_0 /[CBGA] – ([OA]_0 + [GPP]_0) + [CBGA] = (k_2 + k_3) / k_1$

Defining the equilibrium constant K as $k_2/k_1$,
$[OA]_0[GPP]_0 /[CBGA] + [CBGA] = K + k_3 / k_1 + [OA]_0 + [GPP]_0$

Differentiating with respect to $k_1$,

$$\frac{d}{dk_1}\left(\frac{[OA]_0[GPP]_0}{[CBGA]} + [CBGA]\right) = \frac{d}{dk_1}\left(K + \frac{k_3}{k_1} + [OA]_0 + [GPP]_0\right)$$

$$-\frac{[OA]_0[GPP]_0 d[CBGA]}{[CBGA]^2 \quad dk_1} + \frac{d[CBGA]}{dk_1} = -\frac{k_3}{k_1^2}$$

$$\frac{d[CBGA]}{dk_1}\left(1 - \frac{[OA]_0[GPP]_0}{[CBGA]^2}\right) = -\frac{k_3}{k_1^2}$$

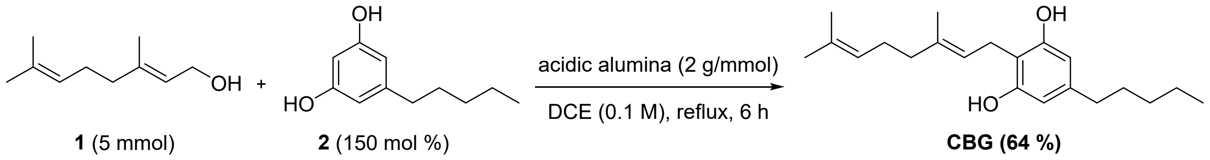
$$\frac{d[CBGA]}{dk_1} = \frac{\frac{k_3}{k_1^2}}{\frac{[OA]_0[GPP]_0}{[CBGA]^2} - 1}$$

$$\frac{d[CBGA]}{dk_1} = \frac{k_3[CBGA]^2}{([OA]_0[GPP]_0 - [CBGA]^2)k_1^2}$$

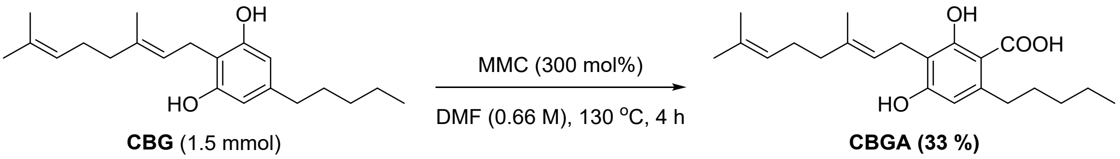
Since $[OA]_0[GPP]_0 - [CBGA]^2$ is positive,

$\frac{d[CBGA]}{dk_1} > 0$ which indicates that the steady-state concentration of CBGA increases as k1 increases.

## Synthesis of cannabigerol (CBG) and CBGA



A round-bottomed 2-neck flask equipped with a magnetic stirrer bar was charged with geraniol (0.867 mL, 5 mmol, 1.0 equiv.), olivetol (1.351 g, 7.5 mmol, 1.5 equiv.), acidic alumina (10 g, 2 g/mmol) and dichloroethane (50 mL, 0.1 M). The flask was fitted with a reflux condenser and heated to reflux temperature. The reaction was monitored by thin-layer chromatography (TLC) until the limiting reagent was completely consumed. After the reaction was complete, the mixture was cooled to room temperature and filtered through a Celite filter. The filter cake was rinsed with ethyl acetate, and the combined filtrate was concentrated under reduced pressure. The concentrated residue was purified by flash column chromatography (*n*-hexane: ethyl acetate = 20:1) to afford a white to yellow solid (1.156 g, 64 % yield); $^1$H Nuclear Magnetic Resonance (NMR) (500 MHz, Chloroform-*d*) $\delta$ 6.25 (2H, s), 5.27 (1H, t, *J* = 7.1 Hz), 5.05 (1H, t, *J* = 6.8 Hz), 3.40 (2H, d, *J* = 7.0 Hz), 2.44 (2H, t, *J* = 7.9 Hz), 2.10 (2H, m), 2.06 (2H, m), 1.81 (3H, s), 1.68 (3H, s), 1.59 (3H, s), 1.56 (2H, m), 1.35 – 1.27 (4H, m), 0.89 (3H, t, *J* = 6.8 Hz). $^{13}$C NMR (126 MHz, Chloroform-*d*) $\delta$ 154.9, 142.9, 139.1, 132.2, 123.9, 121.9, 110.7, 108.5, 39.8, 35.6, 31.6, 3.09, 26.5, 25.8, 22.7, 22.4, 17.8, 16.3, 14.1.



A round-bottomed 2-neck flask equipped with a magnetic stirrer bar was charged with CBG (542.2 mg, 1.5 mmol, 1.0 equiv.) and a 2.0 M solution of methyl magnesium carbonate (MMC, 2.25 mL, 3.0 equiv.) in dimethylformamide. The reaction mixture was heated to 130 °C in an oil bath and stirred for 4 hr. The reaction was monitored by thin-layer chromatography (TLC) until the limiting reagent was completely consumed. After the reaction was complete, the reaction mixture was cooled to room temperature. It was then diluted with a mixture of dichloromethane and methanol (1:1) and carefully quenched with a 10 % hydrochloric acid solution to pH 2. Once the solid was fully dissolved, the reaction mixture was extracted with excess diethyl ether, and the organic layer was evaporated under reduced pressure. The residue was purified using flash column chromatography (*n*-hexane: ethyl acetate = 5:1, with a small amount of formic acid) to afford a yellow solid (178.4 mg, 33 % yield); $^1$H NMR (500 MHz, Chloroform-*d)* δ : 11.88 (s, 1H), 6.29 (s, 1H), 5.29 (t, 1H, *J* = 7.0 Hz), 5.07 (t, 1H, *J* = 6.5

Hz), 3.45 (d, 2H, *J* = 7.0 Hz), 2.88 (t, 2H, *J* = 7.5 Hz), 2.09 (m, 4H), 1.83 (s, 3H), 1.69 (s, 3H), 1.58 (m, 5H), 1.33 (m, 4H), 0.89 (s, 3H). $^{13}$C NMR (126 MHz, Chloroform-*d)* δ : 176.2, 163.7, 160.6, 147.6, 139.3, 132.1, 123.8, 121.4, 111.6, 111.4, 103.2, 39.8, 36.6, 32.1, 31.5, 26.5, 25.8, 22.6, 22.1, 17.8, 16.3, 14.1. Melting point 81.9–82.5 °C. LRMS (ESI-TOF) m/z $[M+H]^+$ calculated for $C_{22}H_{32}O_4$ 361.49; found: 361.43.

CBG was characterized by $^1$H- and $^{13}$C NMR. CBGA was characterized by $^1$H-, $^{13}$C, HSQC, and HMBC NMR. NMR spectra were recorded on a 500 MHz NMR instrument (JEOL, JNM-ECZ500R). Chemical shifts for $^1$H NMR are reported in *δ* units (parts per million, ppm) relative to the residual signal of Chloroform-*d* (7.26 ppm). $^{13}$C NMR spectra are reported in ppm relative to Chloroform-*d* (77.16 ppm), with all measurements obtained with $^1$H decoupling. $^1$H- and $^{13}$C NMR Data of the CBGA compound were matched to previously reported paper[1].

**Scheme S1.** Biological pathways for the production of cannabinoids.

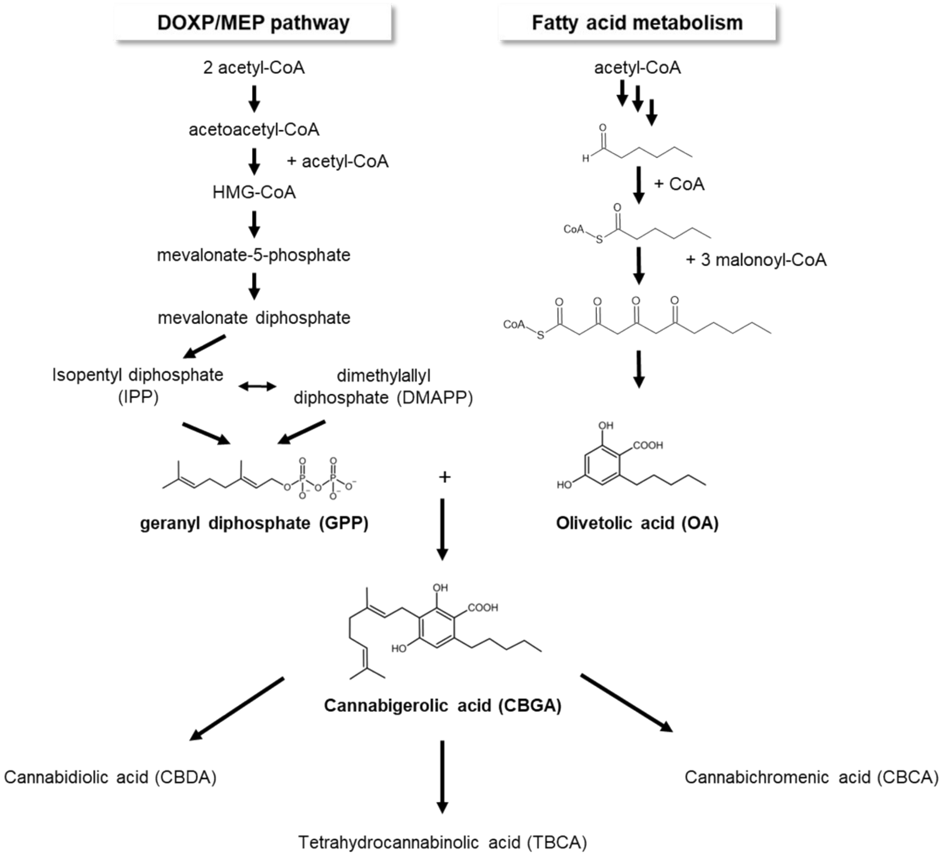


**Figure S1.** Synthesis of CBGA using the wild-type NphB enzyme. 5 μM enzyme and 2 mM substrates (OA and GPP) were incubated at 30 °C for 18 h. The peak for CBGA (~9.6 min) was assigned using the chemically synthesized one (Figure S2). The wild-type enzyme produces CBGA and 2-O-GOA, and the other peak (~10.2 min) was assigned to 2-O-GOA.

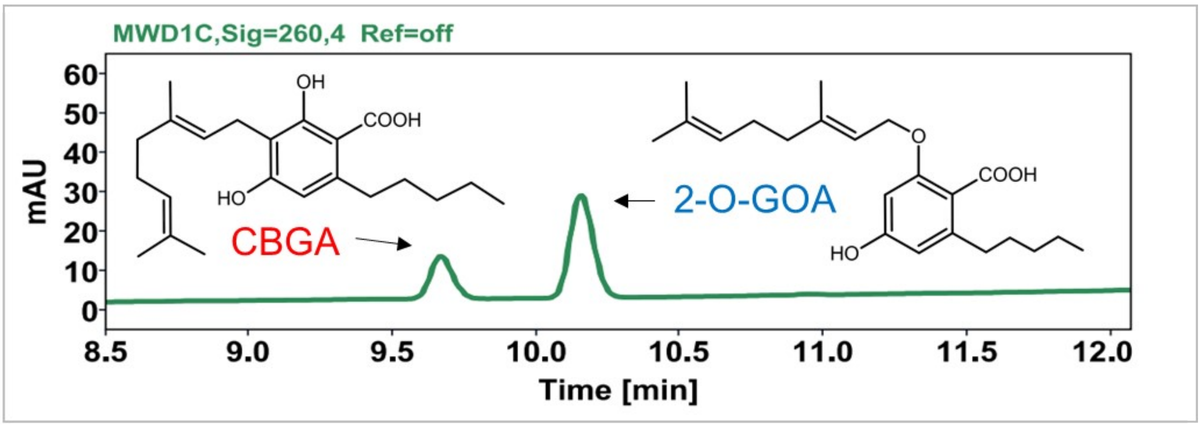
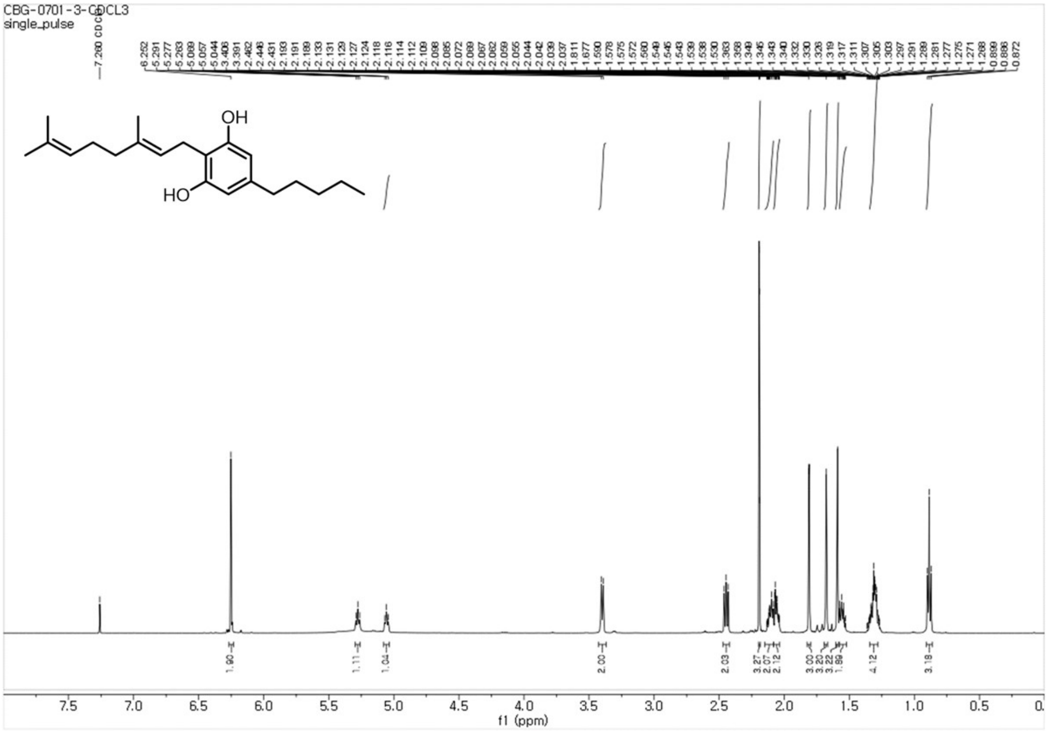
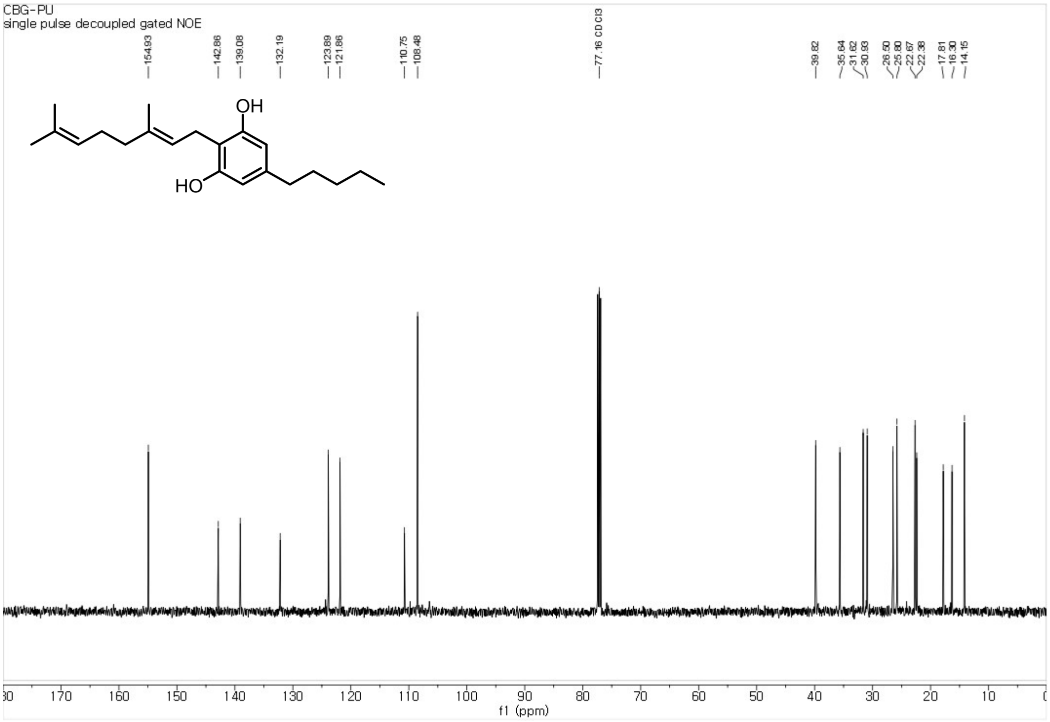


**Figure S2.** NMR spectra

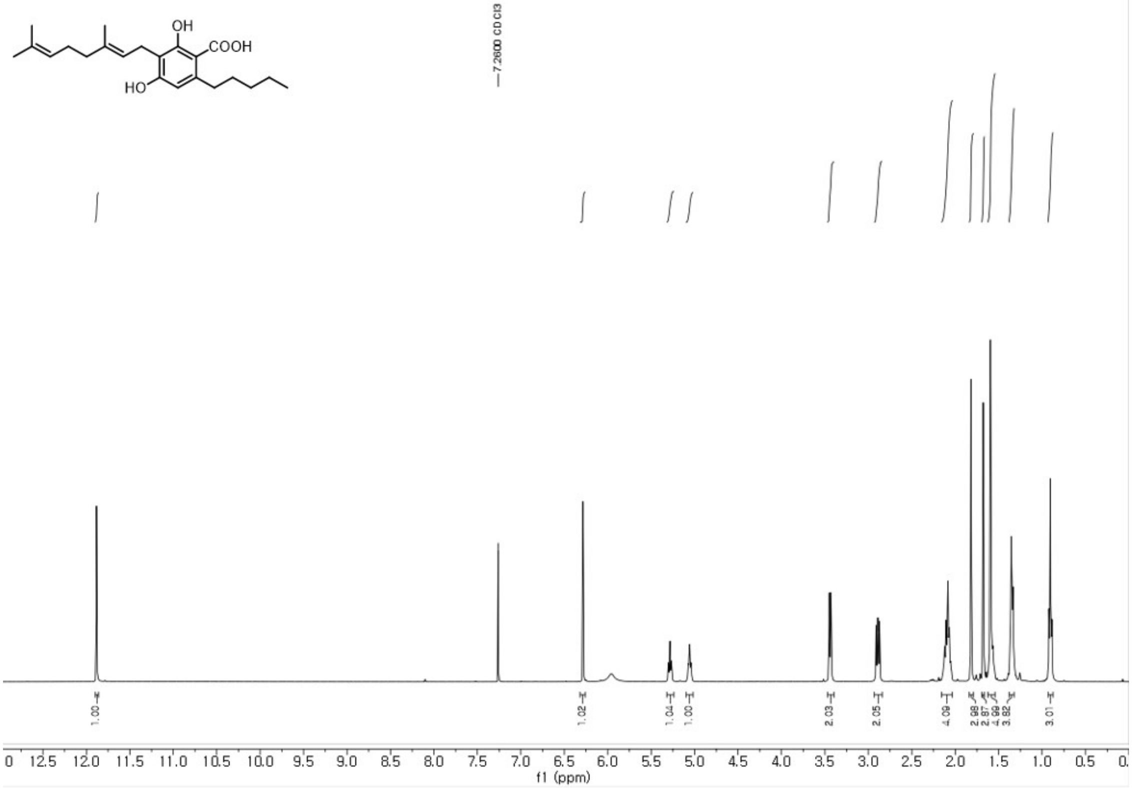
(A) $^1$H NMR of CBG (500 MHz, Chloroform-*d*)



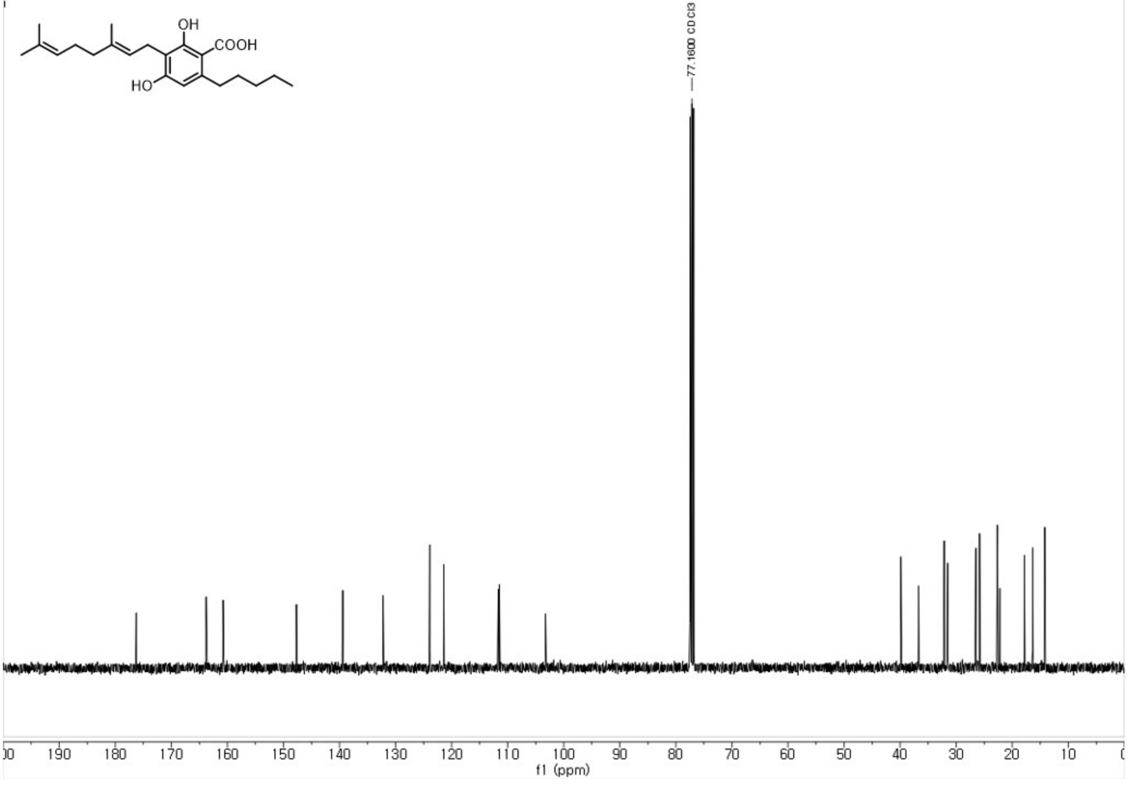
(B) $^{13}$C NMR of CBG (126 MHz, Chloroform-*d*)



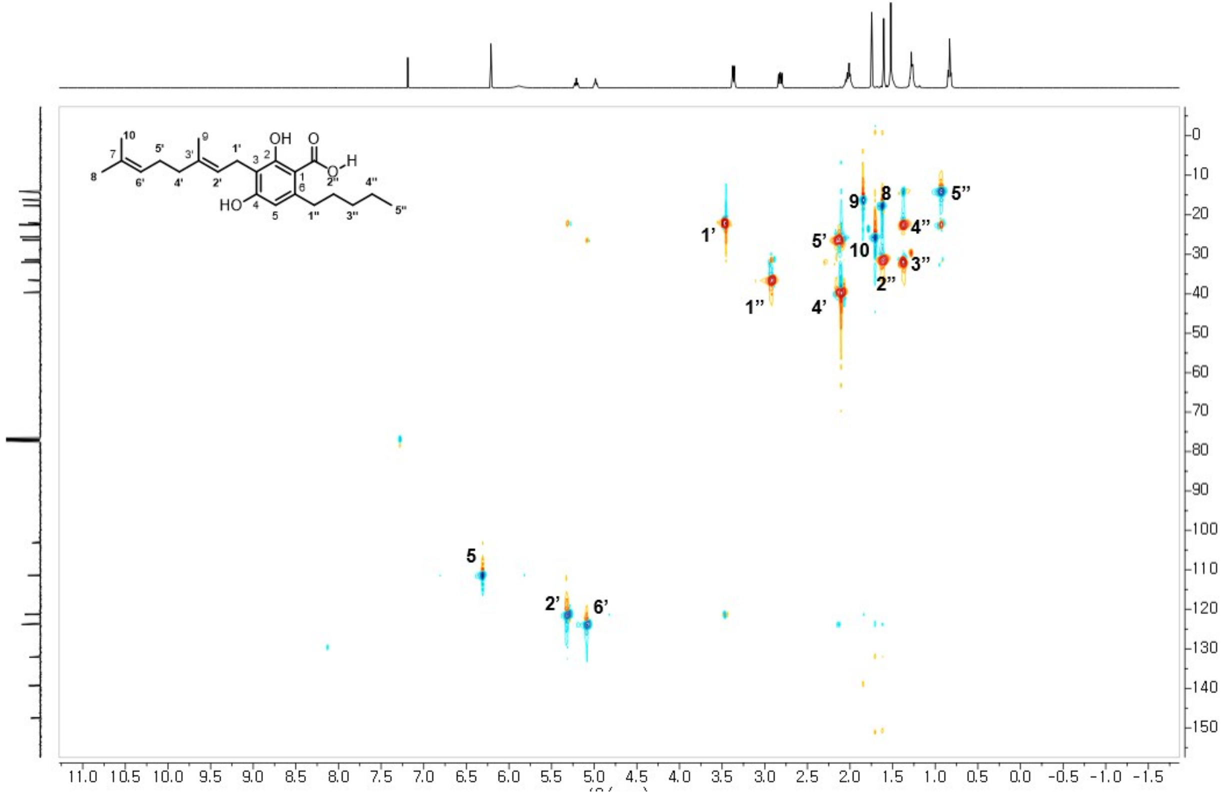
(C) ${}^1$H NMR of CBGA (500 MHz, Chloroform-*d*)



(D) ${}^{13}$C NMR of CBGA (126 MHz, Chloroform-*d*)



(E) HSQC NMR of CBGA (500 MHz, Chloroform-*d*)



(F) HMBC NMR of CBGA (500 MHz, Chloroform-*d*)

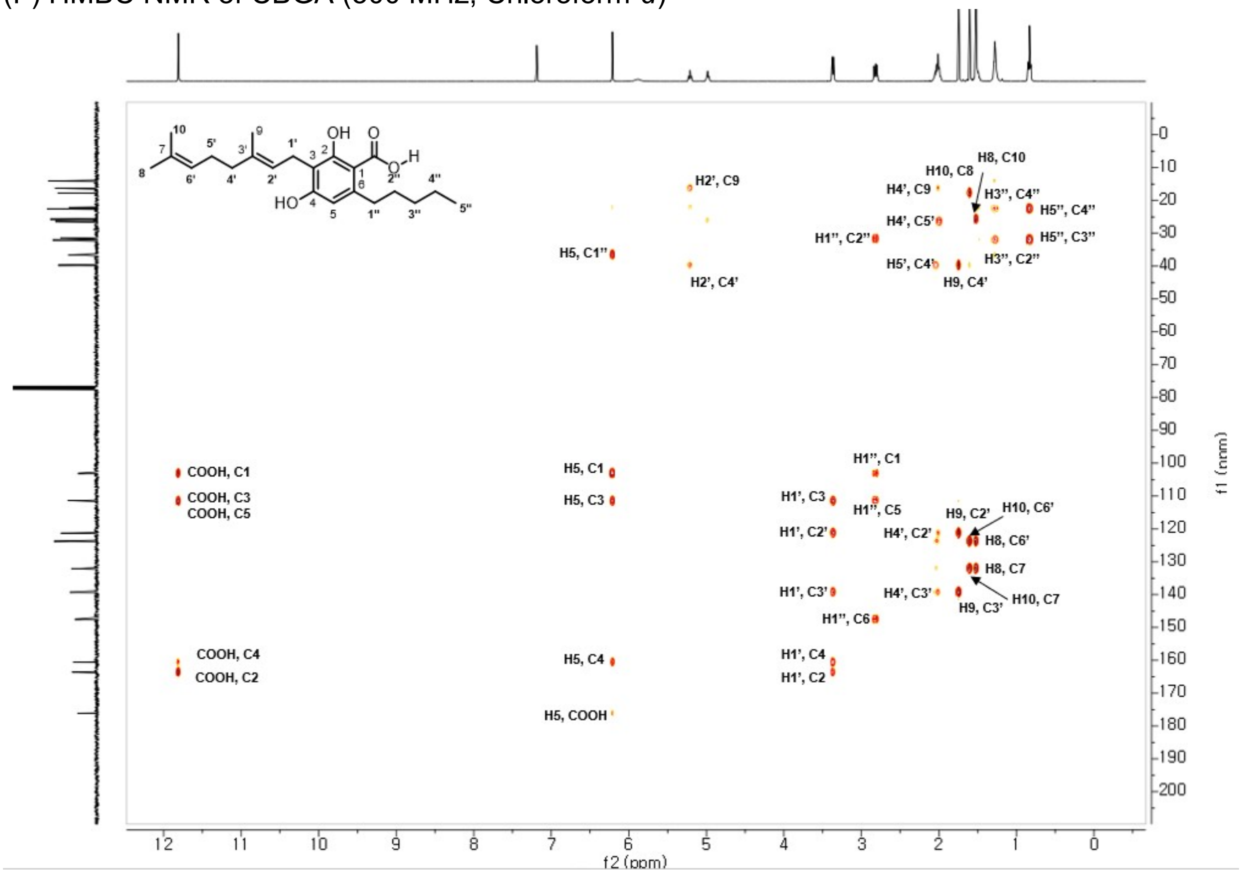


**Figure S3.** Fitting of kinetics data for NphB variants toward OA and GPP

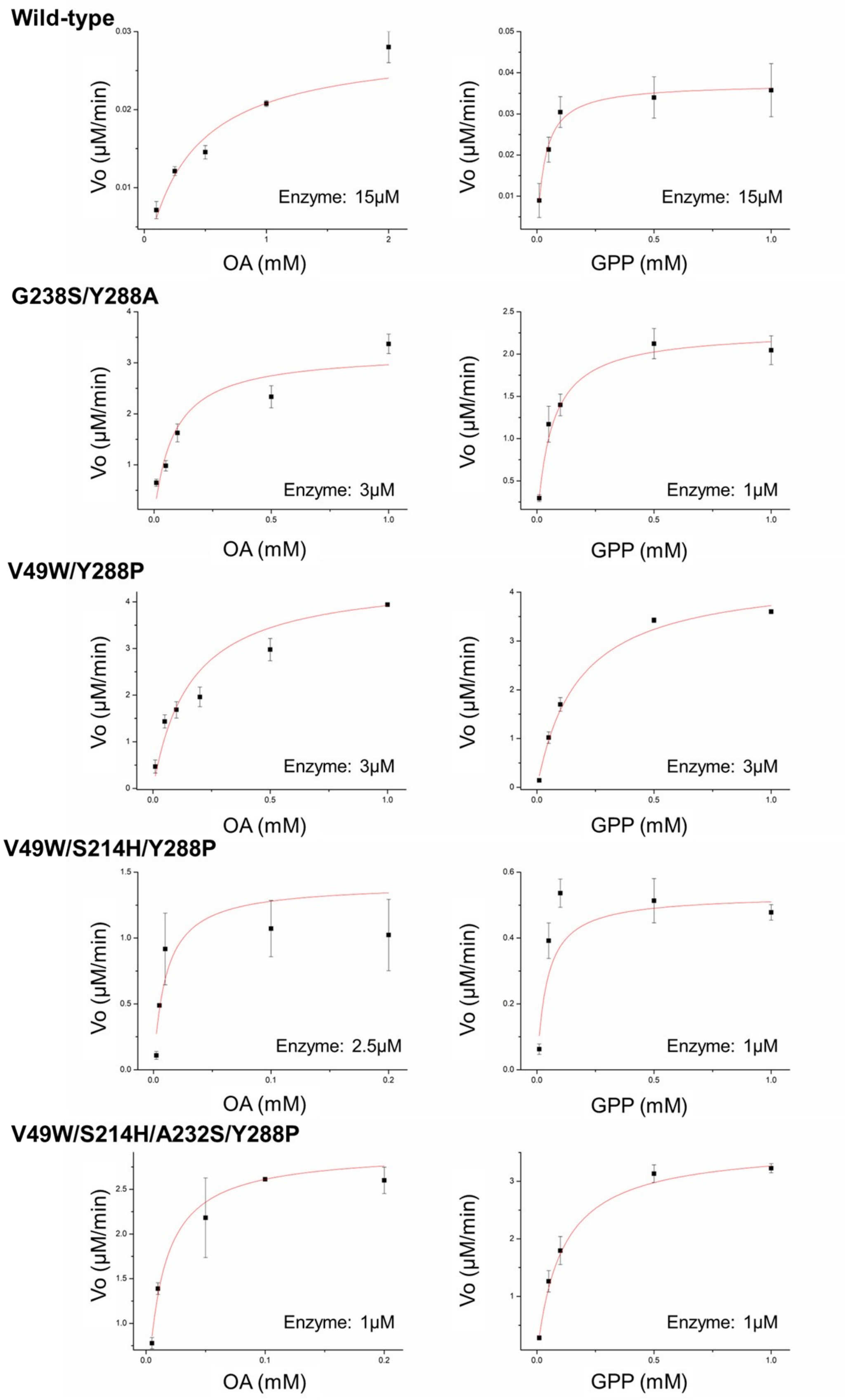


**Figure S4.** Time-course of CBGA concentrations for the reactions catalyzed by NphB variants. 5 μM of each enzyme was incubated with 2 mM OA and GPP at 30°C.

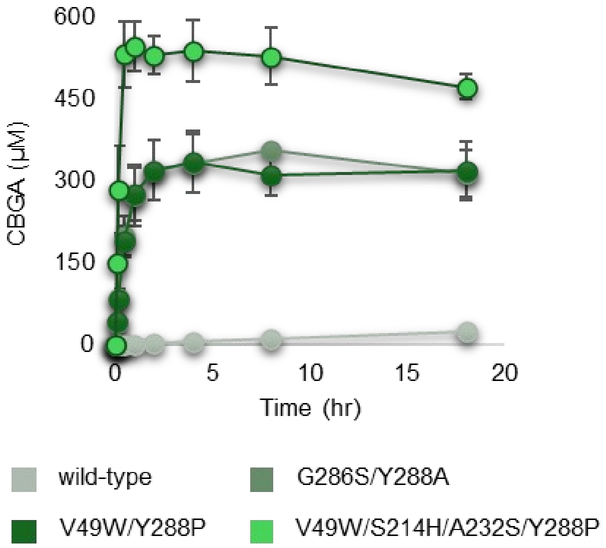


**Figure S5.** Root mean square deviation (RMSD) of backbone atoms during MD simulation of the quadruple variant without substrates.

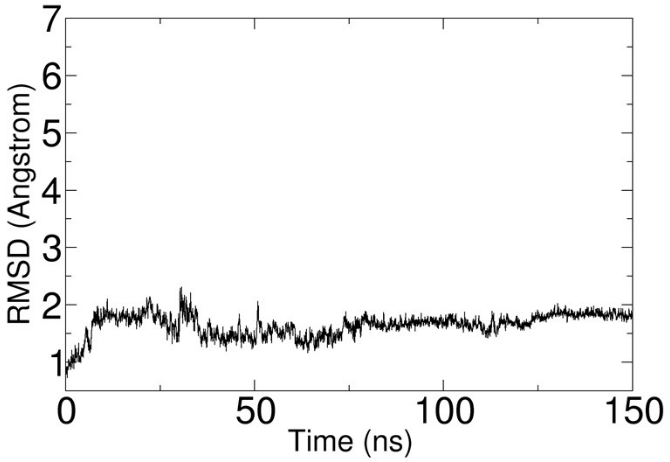


**Figure S6.** RMSD of backbone atoms during MD simulation for the quadruple variant complexed with OA and GSPP.

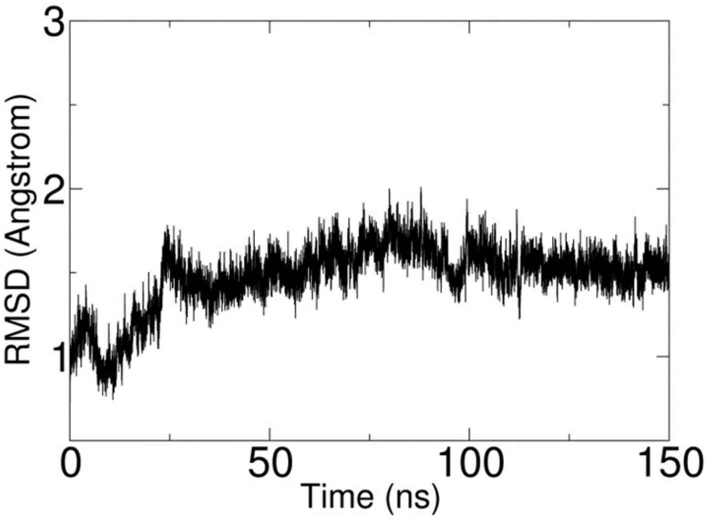


**Table S1.** Synthetic NphB genes used in this study.

| Gene | Sequence (5'→3') |
|---|---|
| Wild-type NphB | CATATGAGTGAAGCGGCGGATGTGGAACGTGTGTATGCGGCAATGGAAGAAGCGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGATAAAATCTATCCGCTGCTGAGCACCTTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGTTGTCTTCAGCATGGCCTCTGGCCGCCATAGTACCGAACTGGATTTTAGTATTTCCGTTCCGACGTCCCACGGTGACCCGTACGCGACCGTGGTTGAAAAAGGCCTGTTTCCGGCCACGGGTCATCCGGTGGATGACCTGCTGGCAGATACCCAAAAACACCTGCCGGTCAGCATGTTTGCTATTGACGGCGAAGTGACCGGCGGTTTCAAGAAAACCTATGCGTTTTTCCCGACCGATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCGTCGATGCCGCCGGCAGTTGCAGAAAATGCTGAACTGTTCGCGCGTTACGGCCTGGATAAAGTTCAGATGACCTCAATGGACTATAAAAAACGCCAAGTCAACCTGTACTTTAGTGAACTGTCCGCCCAGACCCTGGAAGCAGAATCGGTCCTGGCTCTGGTGCGTGAACTGGGCCTGCATGTCCCGAATGAACTGGGTCTGAAATTTTGCAAACGCTCATTCTCGGTGTATCCGACCCTGAACTGGGAAACGGGCAAAATTGATCGTCTGTGTTTCGCAGTGATCTCCAATGACCCGACGCTGGTTCCGAGCTCTGATGAAGGTGACATCGAAAAATTTCACAACTATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACGTACGCTGGTCTACGGTCTGACCCTGAGCCCGAAAGAAGAATATTACAAACTGGGTGCGTATTACCACATTACGGACGTGCAACGCGGTCTGCTGAAAGCATTTGACTCTCTGGAAGATTGACTCGAG |
| NphB M1 | AGTGAAGCGGCGGATGTGGAACGTGTGTATGCGGCAATGGAAGAAGCGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGATAAAATCTATCCGCTGCTGAGCACCTTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGTTTGGTTCAGCATGGCCTCTGGCCGCCATAGTACCGAACTGGATTTTAGTATTTCCGTTCCGACGTCCCACGGTGACCCGTACGCGACCGTGGTTGAAAAAGGCCTGTTTCCGGCCACGGGTCATCCGGTGGATGACCTGCTGGCAGATACCCAAAAACACCTGCCGGTCAGCATGTTTGCTATTGACGGCGAAGTGACCGGCGGTTTCAAGAAAACCTATGCGTTTTTCCCGACCGATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCGTCGATGCCGCCGGCAGTTGCAGAAAATGCTGAACTGTTCGCGCGTTACGGCCTGGATAAAGTTCAGTTTACCTCAATGGACTATAAAAAACGCCAAGTCAACCTGTACTTTAGTGAACTGTCCGCCCAGACCCTGGAAGCAGAATCGGTCCTGGCTCTGGTGCGTGAACTGGGCCTGCATGTCCCGAATGAACTGGGTCTGAAATTTTGCAAACGCTCATTCTCGGTGTATCCGACCCTGAACTGGGAAACGGGCAAAATTGATCGTCTGTGTTTCGCAGTGATCTCCAATGACCCGACGCTGGTTCCGAGCTCTGATGAAGGTGACATCGAAAAATTTCACAACTATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACGTACGCTGGTCTACGGTCTGACCCTGAGCCCGAAAGAAGAATATTACAAACTGGCGGCGGCGTACCACATTACGGACGTGCTGCGCGGTCTGCTGAAAGCATTTGACTCTCTGGAAGATTGA |
| NphB M2 | AGTGAAGCGGCGGATGTGGAACGTGTGTATGCGGCAATGGAAGAAGCGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGATAAAATCTATCCGCTGCTGAGCACCTTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGTTGTCTTCAGCATGGCCTCTGGCCGCCATAGTACCGAACTGGATTTTAGTATTTCCGTTCCGACGTCCCACGGTGACCCGTACGCGACCGTGGTTGAAAAAGGCCTGTTTCCGGCCACGGGTCATCCGGTGGATGACCTGCTGGCAGATACCCAAAAACACCTGCCGGTCAGCATGTTTGCTATTGACGGCGAAGTGACCGGCGGTTTCAAGAAAACCTATGCGTTTTTCCCGACCGATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCGTCGATGCCGCCGGCAGTTGCAGAAAATGCTGAACTGTTCGCGCGTTACGGCCTGGATAAAGTTCAGTTTACCTCAATGGACTATAAAAAACGCCAAGTCAACCTGTACTTTAGTGAACTGTCCGCCCAGACCCTGGAAGCAGAATCGGTCCTGGCTCTGGTGCGTGAACTGGGCCTGCATGTCCCGAATGAACTGGGTCTGAAATTTTGCAAACGCTCATACGCGGTGTATCCGACCCTGAA |

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|---|---|
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| NphB M3 | AGTGAAGCGGCGGATGTGGAACGTGTGTATGCGGCAATGGAAGAAGCGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGATAAAATCTATCCGCTGCTGAGCACCTTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGTTGTCTTCAGCATGGCCTCTGGCCGCCATAGTACCGAACTGGATTTTAGTATTTCCGTTCCGACGTCCCACGGTGACCCGTACGCGACCGTGGTTGAAAAAGGCCTGTTTCCGGCCACGGGTCATCCGGTGGATGACCTGCTGGCAGATACCCAAAAACACCTGCCGGTCAGCATGTTTGCTATTGACGGCGAAGTGACCGGCGGTTTCAAGAAAACCTATGCGTTTTTCCCGACCGATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCGTCGATGCCGCCGGCAGTTGCAGAAAATGCTGAACTGTTCGCGCGTTACGGCCTGGATAAAGTTCAGTTTACCTCAATGGACTATAAAAAACGCCAAGTCAACCTGTACTTTAGTGAACTGTCCGCCCAGACCCTGGAAGCAGAATCGGTCCTGGCTCTGGTGCGTGAACTGGGCCTGCATGTCCCGAATGAACTGGGTCTGAAATTTTGCAAACGCTCAATGCGTGTGTATCCGACCCTGAACTGGGAAACGGGCAAAATTGATCGTCTGTGTTTCACCGTGATCTCCAATGACCCGACGCTGGTTCCGAGCTCTGATGAAGGTGACATCGAAAAATTTCACAACTATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACGTACGCTGGTCTACGGTCTGACCCTGAGCCCGAAAGAAGAATATTACAAACTGGCGGCGTATTACCACATTACGGACGTGCAACGCGGTCTGCTGAAAGCATTTGACTCTCTGGAAGATTGA |
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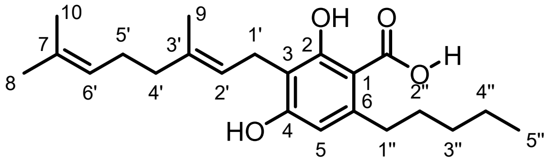
|---|---|
| | GCAGATACCCAAAAACACCTGCCGGTCAGCATGTTTGCTATTGACGGCGAAGTGACCGGCGGTTTCAAGAAAACCTATGCGTTTTTCCCGACCGATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCGTCGATGCCGCCGGCAGTTGCAGAAAATGCTGAACTGTTCGCGCGTTACGGCCTGGATAAAGTTCAGTTTACCTCAATGGACTATAAAAAACGCCAAGTCAACCTGTACTTTAGTGAACTGTCCGCCCAGACCCTGGAAGCAGAATCGGTCCTGGCTCTGGTGCGTGAACTGGGCCTGCATGTCCCGAATGAACTGGGTCTGAAATTTTGCAAACGCTCATTCCGTGTGTATCCGACCCTGAACTGGGAAACGGGCAAAATTGATCGTCTGTGTTTCGCAGTGATCTCCAATGACCCGACGCTGGTTCCGAGCTCTGATGAAGGTGACATCGAAAAATTTCACAACTATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACGTACGCTGGTCTACGGTCTGACCCTGAGCCCGAAAGAAGAATATTACAAACTGCAGGCGTTTTACCACATTACGGACGTGCAACGCGGTCTGCTGAAAGCATTTGACTCTCTGGAAGATTGA |
| NphB M9 | AGTGAAGCGGCGGATGTGGAACGTGTGTATGCGGCAATGGAAGAAGCGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGATAAAATCTATCCGCTGCTGAGCACCTTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGTTATGTTCAGCATGGCCTCTGGCCGCCATAGTACCGAACTGGATTTTAGTATTTCCGTTCCGACGTCCCACGGTGACCCGTACGCGACCGTGGTTGAAAAAGGCCTGTTTCCGGCCACGGGTCATCCGGTGGATGACCTGCTGGCAGATACCCAAAAACACCTGCCGGTCAGCATGTTTGCTATTGACGGCGAAGTGACCGGCGGTTTCAAGAAAACCTATGCGTTTTTCCCGACCGATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCGTCGATGCCGCCGGCAGTTGCAGAAAATGCTGAACTGTTCGCGCGTTACGGCCTGGATAAAGTTCAGTTTACCTCAATGGACTATAAAAAACGCCAAGTCAACCTGTACTTTAGTGAACTGTCCGCCCAGACCCTGGAAGCAGAATCGGTCCTGGCTCTGGTGCGTGAACTGGGCCTGCATGTCCCGAATGAACTGGGTCTGAAATTTTGCAAACGCTCATTCGCGGTGTATCCGACCCTGAACTGGGAAACGGGCAAAATTGATCGTCTGTGTTTCGCAGTGATCTCCAATGACCCGACGCTGGTTCCGAGCTCTGATGAAGGTGACATCGAAAAATTTCACAACTATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACGTACGCTGGTCTACGGTCTGACCCTGAGCCCGAAAGAAGAATATTACAAACTGGCGGCGTATTACCACATTACGGACGTGCTGCGCGGTCTGCTGAAAGCATTTGACTCTCTGGAAGATTGA |
| NphB M10 | AGTGAAGCGGCGGATGTGGAACGTGTGTATGCGGCAATGGAAGAAGCGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGATAAAATCTATCCGCTGCTGAGCACCTTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGTTATTTTCAGCATGGCCTCTGGCCGCCATAGTACCGAACTGGATTTTAGTATTTCCGTTCCGACGTCCCACGGTGACCCGTACGCGACCGTGGTTGAAAAAGGCCTGTTTCCGGCCACGGGTCATCCGGTGGATGACCTGCTGGCAGATACCCAAAAACACCTGCCGGTCAGCATGTTTGCTATTGACGGCGAAGTGACCGGCGGTTTCAAGAAAACCTATGCGTTTTTCCCGACCGATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCGTCGATGCCGCCGGCAGTTGCAGAAAATGCTGAACTGTTCGCGCGTTACGGCCTGGATAAAGTTCAGTTTACCTCAATGGACTATAAAAAACGCCAAGTCAACCTGTACTTTAGTGAACTGTCCGCCCAGACCCTGGAAGCAGAATCGGTCCTGGCTCTGGTGCGTGAACTGGGCCTGCATGTCCCGAATGAACTGGGTCTGAAATTTTGCAAACGCTCATTCTCGGTGTATCCGACCCTGAACTGGGAAACGGGCAAAATTGATCGTCTGTGTTTCGCAGTGATCTCCAATGACCCGACGCTGGTTCCGAGCTCTGATGAAGGTGACATCGAAAAATTTCACAACTATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACGTACGCTGGTCTACGGTCTGACCCTGAGCCCGAAAGAAGAATATTACAAACTGGCGGCGTATTACCACATTACGGACGTGGAACGCGGTCTGCTGAAAGCATTTGACTCTCTGGAAGATTGA |

**Table S2.** Plasmids constructed in this study. The backbone of the plasmids is pET28a.

| Plasmids | Information |
|---|---|
| pSPEL1066 | Wild-type NphB |
| pSPEL1067 | NphB-V49W/Y288P |
| pSPEL1132 | NphB-M1 |
| pSPEL1133 | NphB-M2 |
| pSPEL1134 | NphB-M3 |
| pSPEL1135 | NphB-M4 |
| pSPEL1136 | NphB-M5 |
| pSPEL1137 | NphB-M6 |
| pSPEL1138 | NphB-M7 |
| pSPEL1139 | NphB-M8 |
| pSPEL1140 | NphB-M9 |
| pSPEL1141 | NphB-M10 |
| pSPEL1214 | NphB-F213M |
| pSPEL1215 | NphB-S214R |
| pSPEL1216 | NphB-A232T |
| pSPEL1252 | NphB-S214G |
| pSPEL1253 | NphB-S214C |
| pSPEL1254 | NphB-S214F |
| pSPEL1255 | NphB-S214K |
| pSPEL1256 | NphB-S214H |
| pSPEL1257 | NphB-S214T |
| pSPEL1258 | NphB-S214N |
| pSPEL1265 | NphB-S214V |
| pSPEL1266 | NphB-S214A |
| pSPEL1269 | NphB-S214D |
| pSPEL1296 | NphB-S214R/V288V |
| pSPEL1297 | NphB-S214R/V288G |
| pSPEL1299 | NphB-S214R/V288A |
| pSPEL1300 | NphB-S214R/V288P |
| pSPEL1306 | NphB-S214H/V288V |
| pSPEL1307 | NphB-S214H/V288G |
| pSPEL1309 | NphB-S214H/V288A |
| pSPEL1310 | NphB-S214H/V288P |
| pSPEL1318 | NphB-G286S/Y288A |
| pSPEL1337 | NphB-V49W/S214H/V288P |
| pSPEL1352 | NphB-S214H/V288P/A232S |
| pSPEL1353 | NphB-S214H/V288P/A232T |
| pSPEL1355 | NphB-S214H/V288P/A232N |
| pSPEL1358 | NphB-V49W/S214H/A232S/V288P |

**Table S3**. NMR peak assignments

(A) Structure of CBGA with atom numbering and $^1H/^{13}C$ NMR assignments



| No. | $^{13}C$ (ppm) | $^1H$ (ppm) |
|---|---|---|
| 1 | 103.2 | - |
| 2 | 163.7 | - |
| 3 | 111.4 | - |
| 4 | 160.6 | - |
| 5 | 111.6 | 6.29 (s, 1H) |
| 6 | 147.6 | - |
| 7 | 132.1 | - |
| 8 | 17.8 | 1.58 (m, 2H) |
| 9 | 16.3 | 1.83 (s, 3H) |
| 10 | 25.8 | 1.69 (s, 3H) |
| 1' | 22.1 | 3.45 (d, 2H, *J* = 7.0 Hz) |
| 2' | 121.4 | 5.29 (t, 1H, *J* = 7.0 Hz) |
| 3' | 139.3 | - |
| 4' | 39.8 | 2.09 (m, 2H) |
| 5' | 26.5 | 2.09 (m, 2H) |
| 6' | 123.8 | 5.07 (t, 1H, *J*= 6.5 Hz) |
| 1'' | 36.6 | 2.88 (t, 2H, *J* = 7.5 Hz) |
| 2'' | 31.5 | 1.58 (m, 3H) |
| 3'' | 32.1 | 1.33 (m, 4H) |
| 4'' | 22.6 | 1.33 (m, 4H) |
| 5'' | 14.1 | 0.89 (s, 3H) |
| COOH | 176.2 | 11.88 (s, 1H) |

(B) Chemical shifts of 1D NMR signals and 2D NMR correlations of CBGA

| Atom | δ (ppm) | HSQC | HMBC |
|---|---|---|---|
| C1 | 103.2 | - | 1”, 5, COOH |
| C2 | 163.7 | - | 1’, COOH |
| C3 | 111.4 | - | 5, 1”, COOH |
| C4 | 160.6 | - | 5, 1’, COOH |
| C5 | 111.6 | 5 | 1”, COOH |
| H5 | 6.29 (s, 1H) | 5 | 1, 3, 4, 1”, COOH |
| C6 | 147.6 | - | 1” |
| C7 | 132.1 | - | 8, 10 |
| C8 | 17.8 | 8 | 10 |
| H8 | 1.58 (m, 2H) | 8 | 7, 10, 6’ |
| C9 | 16.3 | 9 | 2’, 4’ |
| H9 | 1.83 (s, 3H) | 9 | 2’, 3’, 4’ |
| C10 | 25.8 | 10 | 8 |
| H10 | 1.69 (s, 3H) | 10 | 7, 8, 6’ |
| C1’ | 22.1 | 1’ | - |
| H1’ | 3.45 (d, 2H, *J* = 7.0 Hz) | 1’ | 2, 3, 4, 2’, 3’, |
| C2’ | 121.4 | 2’ | 9, 1’, 4’ |
| H2’ | 5.29 (t, 1H, *J* = 7.0 Hz) | 2’ | 9, 4’, |
| C3’ | 139.3 | - | 9, 1’, 4’ |
| C4’ | 39.8 | 4’ | 9, 2, 5’ |
| H4’ | 2.09 (m, 2H) | 4’ | 9, 2’, 3’, 5’ |
| C5’ | 26.5 | 5’ | 4’ |
| H5’ | 2.09 (m, 2H) | 5’ | 4’ |
| C6’ | 123.8 | 6’ | 8, 10 |
| H6’ | 5.07 (t, 1H, *J* = 6.5 Hz) | 6’ | - |
| C1” | 36.6 | 1” | 5 |
| H1” | 2.88 (t, 2H, *J* = 7.5 Hz) | 1” | 1, 5, 6, 2” |
| C2” | 31.5 | 2” | 1”, 3” |
| H2” | 1.58 (m, 3H) | 2” | - |
| C3” | 32.1 | 3” | 4” |
| H3” | 1.33 (m, 4H) | 3” | 2”,4” |
| C4” | 22.6 | 4” | 3”, 5” |
| H4” | 1.33 (m, 4H) | 4” | 3” |
| C5” | 14.1 | 5” | - |
| H5” | 0.89 (s, 3H) | 5” | 3”, 4” |
| COOH | 176.2 | - | 5 |
| COOH | 11.88 (s, 1H) | - | 1, 2, 3, 4, 5, |

**Table S4.** Kinetic parameters of NphB variants obtained using a three-dimensional Michaelis-Menten equation

| | $k_{cat}$ (min$^{-1}$) | $K_m$ (OA) (mM) | $K_m$ (GPP) (mM) |
|---|---|---|---|
| **WT** | 0.0028 ± 0.00028 | 0.604 ± 0.1679 | 0.0208 ± 0.00651 |
| **G286S/Y288A** | 4.39 ± 0.935 | 2.26 ± 0.804 | 0.0386 ± 0.00762 |
| **V49W/Y288P** | 1.60 ± 0.112 | 0.204 ± 0.0425 | 0.130 ± 0.0294 |
| **V49W/S214H/Y288P** | 0.494 ± 0.0460 | 0.0336 ± 0.01545 | 0.0225 ± 0.01085 |
| **V49W/S214H/A232S/Y288P** | 3.04 ± 0.137 | 0.0129 ± 0.00317 | 0.0646 ± 0.01490 |

The three-dimensional Michaelis-Menten equation is shown below:

$$v = \frac{kcat\ \times [Enzyme]}{\left(1 + \frac{Km\ (OA)}{[OA]}\right) \times (1 + \frac{Km\ (GPP)}{[GPP]})}$$

## References

1. L. Marchetti, V. Brighenti, M. C. Rossi, J. Sperlea, F. Pellati and D. Bertelli, *Molecules*, 2019, **24**.