Supplementary Information

FUNCTIONAL CHARACTERIZATION OF THE PROMISCUOUS PRENYLTRANSFERASE RESPONSIBLE FOR FURAQUINOCIN BIOSYNTHESIS: IDENTIFICATION OF A PHYSIOLOGICAL POLYKETIDE SUBSTRATE AND ITS PRENYLATED REACTION PRODUCTS

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Running head: Prenyltransferase in furaquinocin biosynthesis

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^{1}H-, ^{13}C-NMR and HR-MS data of the prenylated products.

The structures were analyzed by their mass spectral data [HRMS (ESI^{-}); JEOL JMS-T100LC] and nuclear magnetic resonance (NMR) spectral data (600 MHz, JEOL ECA-600).

(*E*)-3-(3,7-dimethylocta-2,6-dienyl)-2,5,7-trihydroxynaphthalene-1,4-dione 5 was converted from flaviolin **1** by recombinant Fur7.

HRMS (ESI^{-}) calcd. for $C_{20}H_{21}O_5$ [M−H]$^{-}$, 341.13890; found 341.13531. ^{1}H NMR (DMSO-*d6*) δ: 1.47 (s, 3H, Me-10'), 1.53 (s, 3H, Me-8'), 1.64 (s, 3H, Me-9'), 1.85 (m, 2H, H-4'), 1.95 (m, 2H, H-5'), 3.01 (d, *J* = 6.8 Hz, 2H, H-1'), 4.98 (t, *J* = 6.8 Hz, 1H, H-6'), 5.07 (t, *J* = 6.8 Hz, 1H, H-2'), 6.41 (s, 1H, H-6), 6.81 (s, 1H, H-8), 13.60 (s, 1H, C-5-OH), ^{13}C NMR (DMSO-*d6*) δ: 16.6 (C-9'), 18.1 (C-10'), 22.0 (C-1'), 26.1 (C-8'), 26.8 (C-5'), 39.9 (C-4'), 107.6 (C-8), 108.7 (C-4a), 108.7 (C-6), 120.1 (C-3), 122.8 (C-2'), 124.8 (C-6'), 131.3 (C-7'), 132.4 (C-8a), 134.7 (C-3'), 162.2 (C-2), 162.9 (C-7), 163.3 (C-5), 183.2 (C-1), 189.9 (C-4).

3-(3,7-dimethylocta-1,6-dien-3-yl)-2,5,7-trihydroxynaphthalene-1,4-dione 6 was converted from **1** by recombinant Fur7.

HRMS (ESI^{-}) calcd. for $C_{20}H_{21}O_5$ [M−H]$^{-}$, 341.13890; found 341.14055. ^{1}H NMR (DMSO-*d6*) δ: 1.41 (s, 3H, H-7'), 1.44 (s, 3H, H-10'), 1.49 (s, 3H, H-7'), 1.72 (m, 1H, H-2'), 1.83 (m, 2H, H-3'), 1.99 (m, 1H, H-2'), 4.76 (d, *J* = 10.3 Hz, 1H, H-9'),4.82 (d, *J* = 18.0 Hz, 1H, H-9'), 4.98 (t, *J* = 6.8 Hz, 1H, H-4'), 6.22 (dd, *J* = 10.3, 18.0 Hz, 1H, H-8'), 6.45 (s, 1H, H-6), 6.84 (s, 1H, H-8), 13.18 (s, 1H, C-5-OH), ^{13}C NMR (DMSO-*d6*) δ: 17.9 (C-10'), 24.1 (C-5'), 25.9 (C-8'), 26.5 (C-9'), 40.8 (C-4'), 44.2, (C-3'), 107.0 (C-8), 108.7 (C-6), 109.0 (C-4a), 109.2 (C-1'), 124.0 (C-3), 125.6 (C-6'), 130.8 (C-7'), 131.7 (C-8a), 149.2 (C-2'), 159.8 (C-2), 163.0 (C-7), 163.4 (C-5), 182.3 (C-1), 190.0 (C-4).

2,5,7-trihydroxy-3-(3-methylbut-2-en-1-yl)naphthalene-1,4-dione was converted from **1** by recombinant Fur7.

^{1}H NMR (CD_3OD) δ: 1.64 (s, 3H, Me-5'), 1.74 (s, 3H, Me-4'), 3.17 (d, *J* = 6.8 Hz, 2H, H-1'), 5.14 (m, 1H, H-2'), 6.46 (s, 1H, H-6), 6.96 (s, 1H, H-8)

2,5,7-trihydroxy-3-(2-methylbut-3-en-2-yl)naphthalene-1,4-dione was converted from **1** by recombinant Fur7.

^{1}H NMR (CD_3OD) δ: 1.52 (s, 6H, Me-1', Me-5'), 4.82 (d, J = 10.9 Hz, 1H, H-4'), 4.90 (d, 16.5 Hz, 1H, H-4'), 6.24 (dd, *J* = 10.9, 16.5 Hz, 2H, H-3'), 6.47 (s, 1H, H-6), 6.96 (s, 1H, H-8)

1-*O*-geranyl-1,3-DHN was converted from 1,3-DHN by recombinant Fur7.

HRMS (ESI$^-$) calcd. for $C_{20}H_{23}O_2$ $[M-H]^-$, 295.16980; found 295.17000. ^{1}H NMR (CD_3OD) δ:1.59 (s, 3H, Me-10'), 1.63 (s, 3H, Me-8'), 1.77 (s, 3H, Me-9'), 2.03 (m, 2H, H-4'), 2.10 (m, 2H, H-5'), 4.67 (d, J=6.9 Hz, 2H, H-1'), 5.10 (m, 1H, H-2'), 5.55 (m, 1H, H-6'), 6.47 (s, 1H, H-2), 6.65 (s, 1H, H-4), 7.16 (t, *J*=7.6 Hz, 1H, H-7), 7.31 (t, *J*=7.6 Hz, 1H, H-6), 7.52 (d, *J*=8.3 Hz, 1H, H-5), 8.01 (d, *J*=8.3 Hz, 1H, H-8)

1-geranyl-2,7-DHN was converted from 2,7-DHN by recombinant Fur7.

HRMS (ESI$^-$) calcd. for $C_{20}H_{23}O_2$ $[M-H]^-$, 295.16980; found 295.16599. ^{1}H NMR (CD_3OD) δ: 1.50 (s, 3H, Me-10), 1.53 (s, 3H, Me-8'), 1.85 (s, 3H, Me-9'), 1.95 (m, 2H, H-4'), 2.03 (m, 2H, H-5'), 3.62 (d, *J* = 6.2 Hz, 2H, H-1'), 5.01 (m, 1H, H-6'), 5.15 (m, 1H, H-2'), 6.81 (d, *J* = 8.9 Hz, 1H, H-6), 6.86 (d, *J* = 8.9 Hz, 1H, H-3), 7.10 (s, 1H, H-8), 7.42 (d, *J* = 8.9 Hz, 1H, H-5), 7.55 (d, *J* = 8.9 Hz, 1H, H-4)

2-geranyl-resveratrol was converted from resveratrol by recombinant Fur7.

^{1}H NMR (DMSO-*d6*) δ: 1.40 (s, 3H, Me-10"), 1.46 (s, 3H, Me-8"), 1.73 (s, 3H, Me-9"), 1.84 (m, 2H, H-4"), 1.91 (m, 2H, H-5"), 3.22 (d, *J* = 6.2 Hz, 2H, H-1"), 4.91 (m, 2H, H-2", H-6"), 6.16 (s, 1H, H-4), 6.43 (s, 2H, H-6), 6.70 (d, *J* = 8.3 Hz, 2H, H-3', H-5'), 6.75 (d, *J* = 15.8 Hz, 1H, H-β), 6.96 (d, *J* = 15.8 Hz, 1H, H-α), 7.28 (d, *J* = 8.3 Hz, 2H, H-2', H-6')

Legends for Supplementary Figure

SUPPLEMENTAL FIGURE S1. **Michaelis-Menten plot and Lineweaver-Burk plot of Fur7 reaction with 2 mM of GPP and varied [Fur-P1].**
Fixed concentration of GPP (2 mM) and various concentration of Fur-P1 (0.03 − 0.7 mM). Values are expressed as the means ± S.D. of three independent experiments.

SUPPLEMENTAL FIGURE S2. **Michaelis-Menten plot and Lineweaver-Burk plot of Fur7 reaction with 0.5 mM of Fur-P1 and varied [GPP].**
Fixed concentration of Fur-P1 (0.5 mM) and various concentration of GPP (0.05 − 0.5 mM). Values are expressed as the means ± S.D. of three independent experiments.

SUPPLEMENTAL FIGURE S3. **Michaelis-Menten plot and Lineweaver-Burk plot of Fur7 reaction with 2 mM of GPP and varied [flaviolin].**
Fixed concentration of GPP (2 mM) and various concentration of flaviolin (0.02 – 1.0 mM). Values are expressed as the means ± S.D. of three independent experiments.

SUPPLEMENTAL FIGURE S4. **Michaelis-Menten plot and Lineweaver-Burk plot of Fur7 reaction with 0.5 mM of flaviolin and varied [GPP].**
Fixed concentration of flaviolin (0.5 mM) and various concentration of GPP (0.05 – 1.0 mM). Values are expressed as the means ± S.D. of three independent experiments.

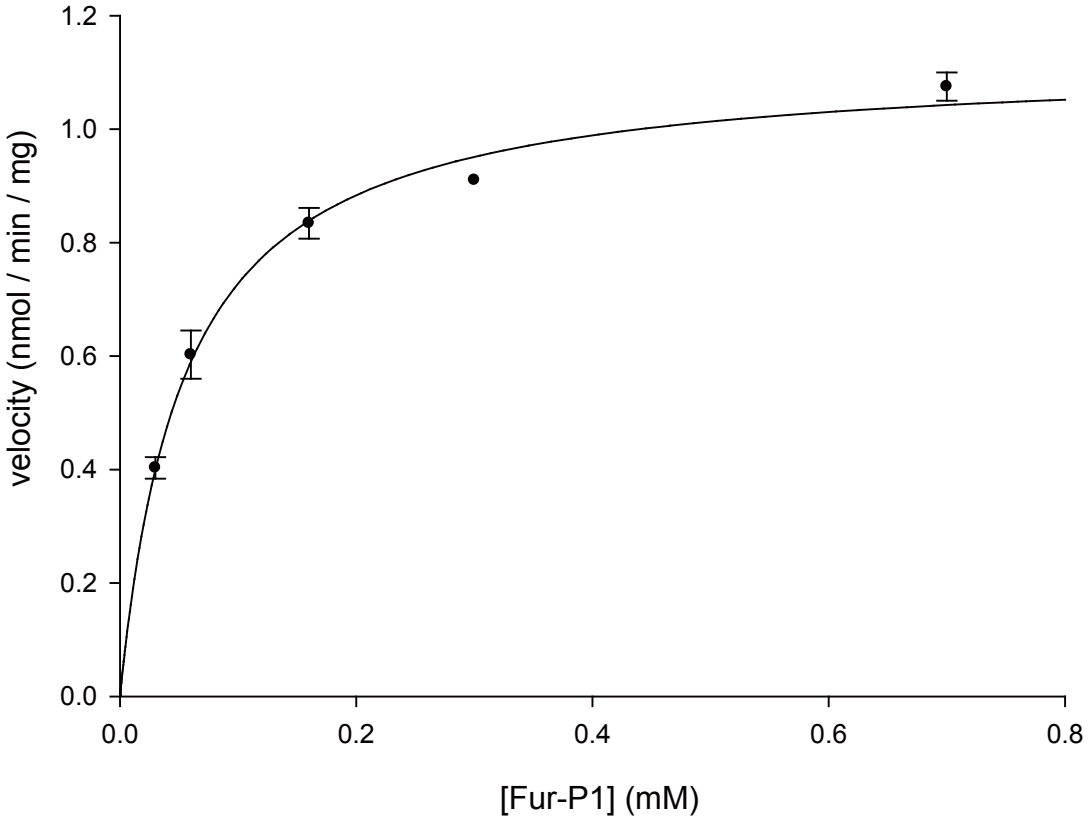
SUPPLEMENTAL FIGURE S5. **Michaelis-Menten plot and Lineweaver-Burk plot of Fur7 reaction with 4 mM of DMAPP and varied [flaviolin].**
Fixed concentration of DMAPP (4 mM) and various concentration of flaviolin (0.1 – 5.0 mM). Values are expressed as the means ± S.D. of three independent experiments.

SUPPLEMENTAL FIGURE S6. **Michaelis-Menten plot and Lineweaver-Burk plot of Fur7 reaction with 2 mM of flaviolin and varied [DMAPP].**

Fixed concentration of flaviolin (2 mM) and various concentration of DMAPP (0.1 – 5.0 mM). Values are expressed as the means ± S.D. of three independent experiments.

SUPPLEMENTAL FIGURE S7. **Michaelis-Menten plot and Lineweaver-Burk plot of Fur7 reaction with 2 mM of GPP and varied [1,3-DHN].**
Fixed concentration of GPP (2 mM) and various concentration of 1,3-DHN (0.1 – 5.0 mM). Values are expressed as the means ± S.D. of three independent experiments.

SUPPLEMENTAL FIGURE S8. **Michaelis-Menten plot and Lineweaver-Burk plot of Fur7 reaction with 5 mM of 1,3-DHN and varied [GPP].**
Fixed concentration of 1,3-DHN (5 mM) and various concentration of GPP (0.05 – 1.0 mM). Values are expressed as the means ± S.D. of three independent experiments.


velocity (nmol / min / mg)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
[Fur-P1] (mM)

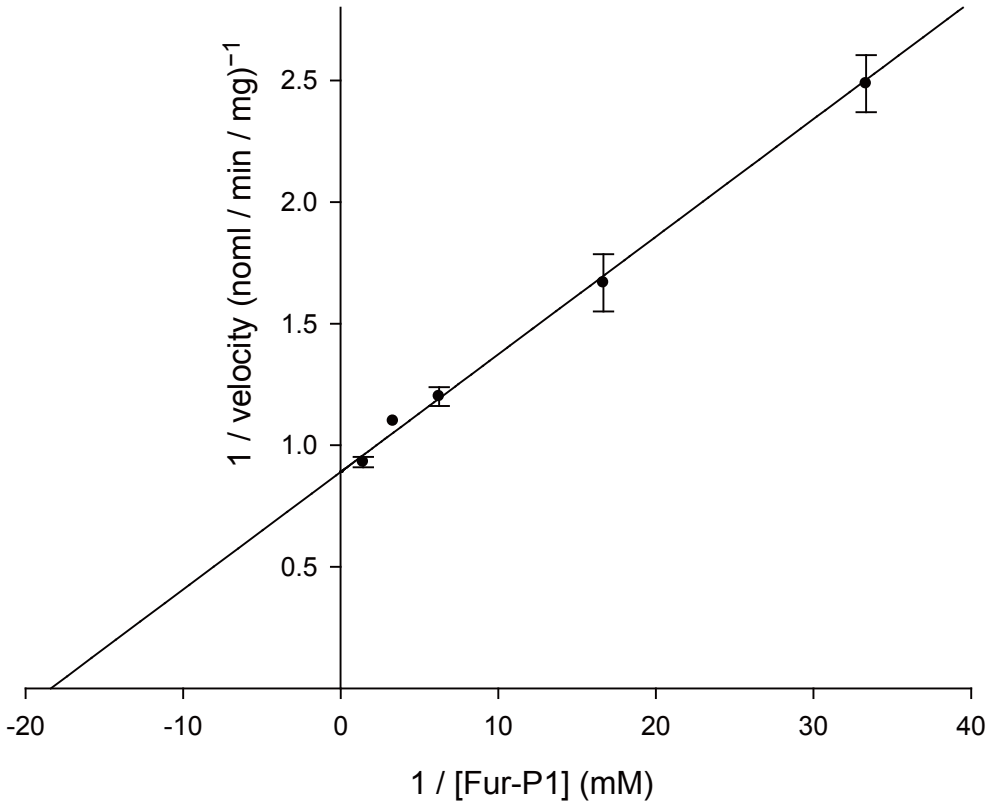
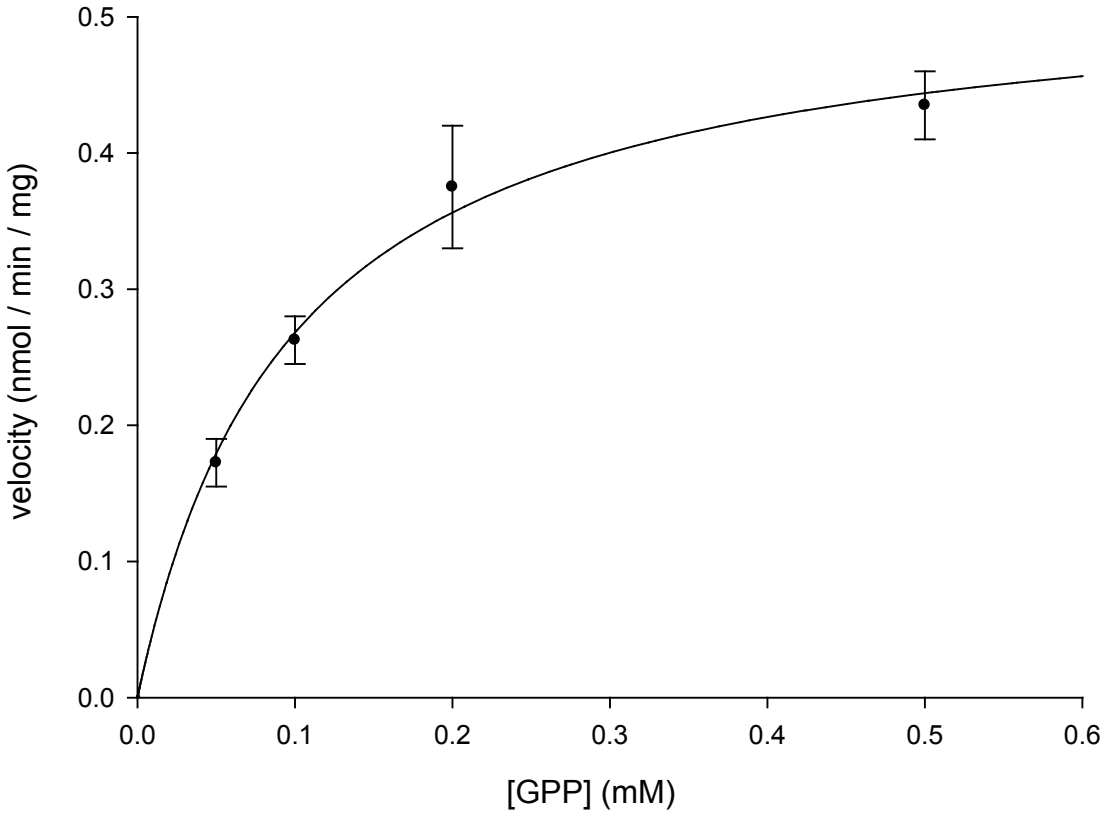

1 / velocity (noml / min / mg)$^{-1}$
2.5
2.0
1.5
1.0
0.5
-20
-10
0
10
20
30
40
1 / [Fur-P1] (mM)

Fig. S1


velocity (nmol / min / mg)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
[GPP] (mM)

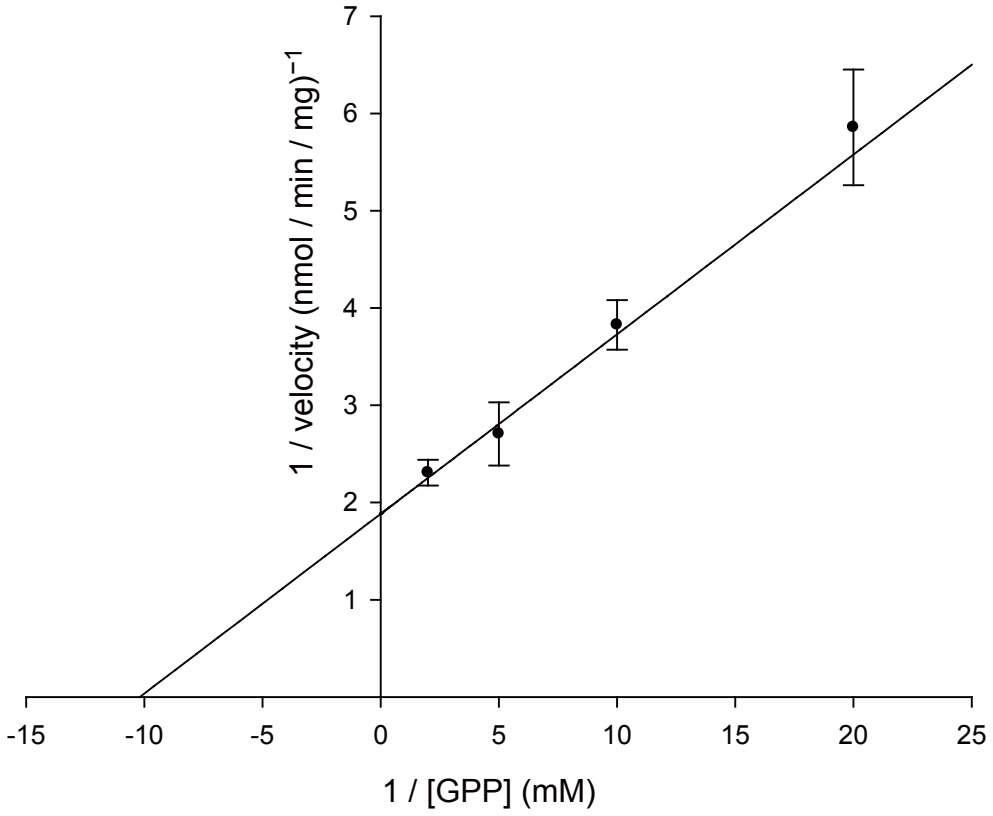
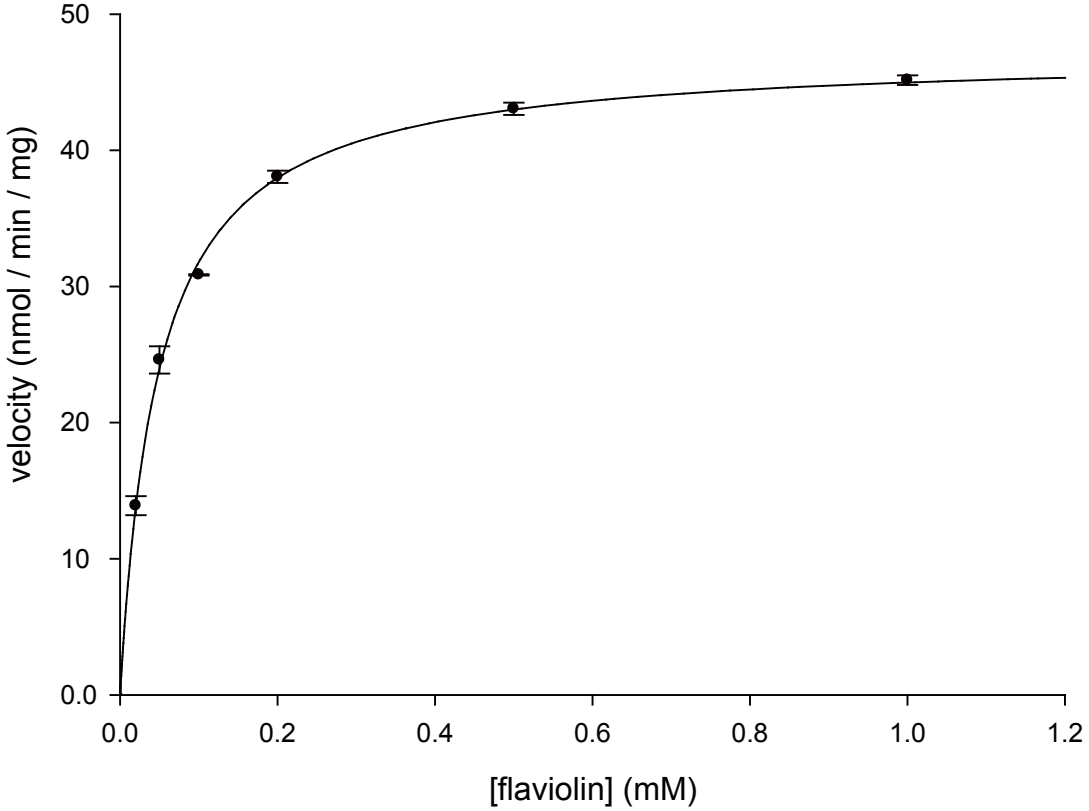

1 / velocity (nmol / min / mg)$^{-1}$
1
2
3
4
5
6
7
-15
-10
-5
0
5
10
15
20
25
1 / [GPP] (mM)

Fig. S2


velocity (nmol / min / mg)
50
40
30
20
10
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
[flaviolin] (mM)

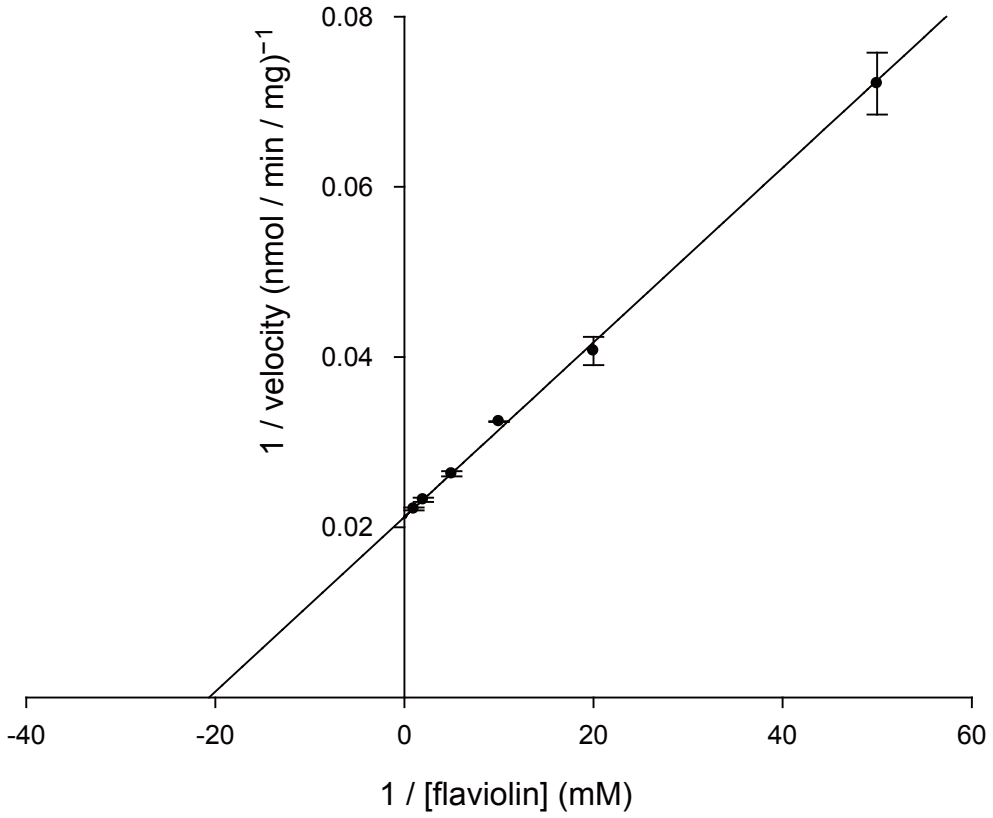
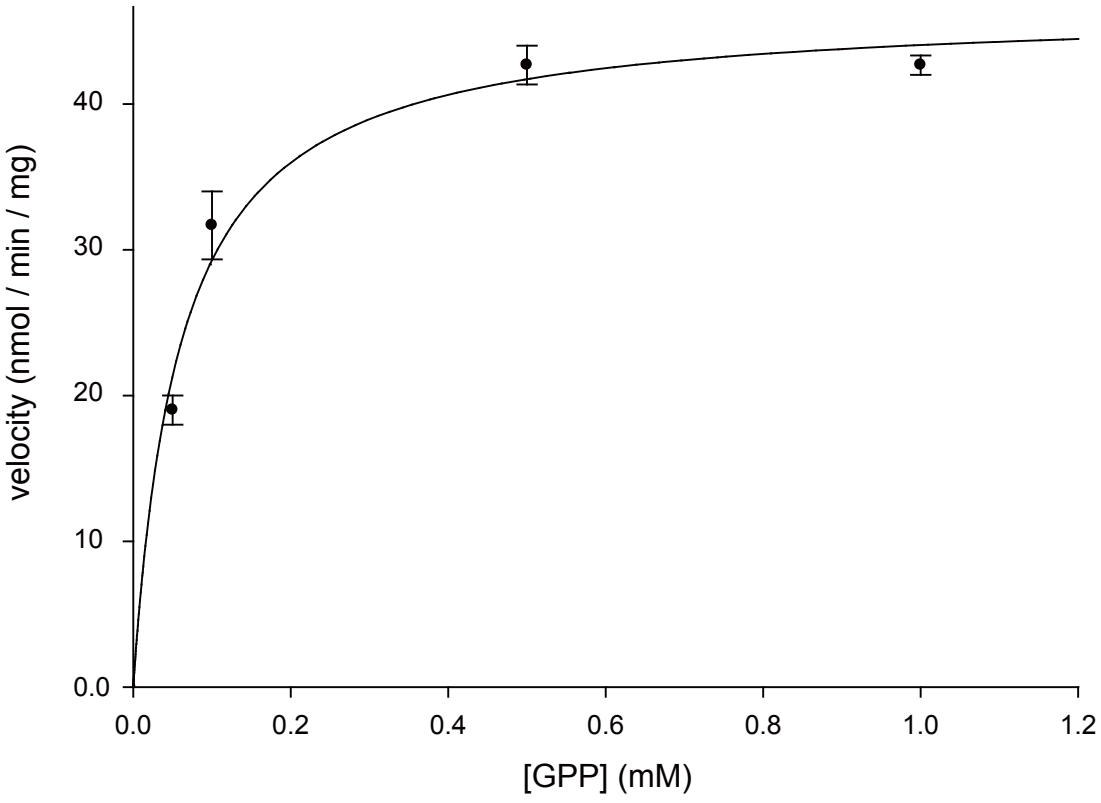

1 / velocity (nmol / min / mg)$^{-1}$
0.08
0.06
0.04
0.02
-40
-20
0
20
40
60
1 / [flaviolin] (mM)

Fig. S3


velocity (nmol / min / mg)
0.0
10
20
30
40
0.0
0.2
0.4
0.6
0.8
1.0
1.2
[GPP] (mM)

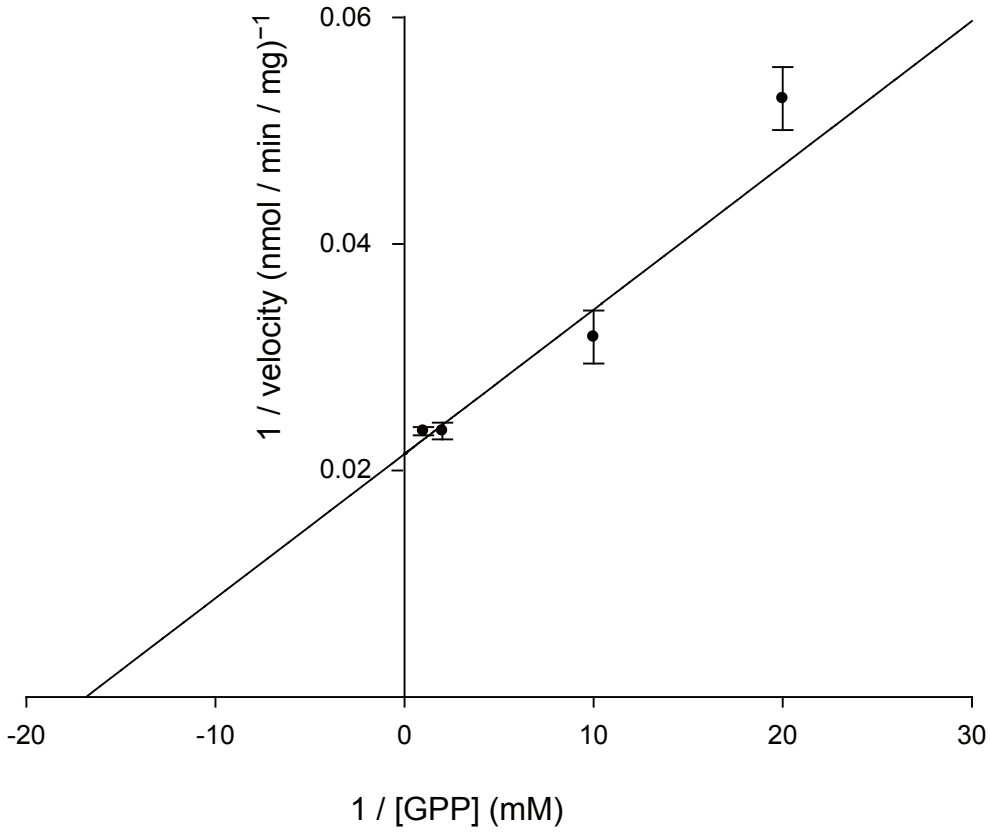
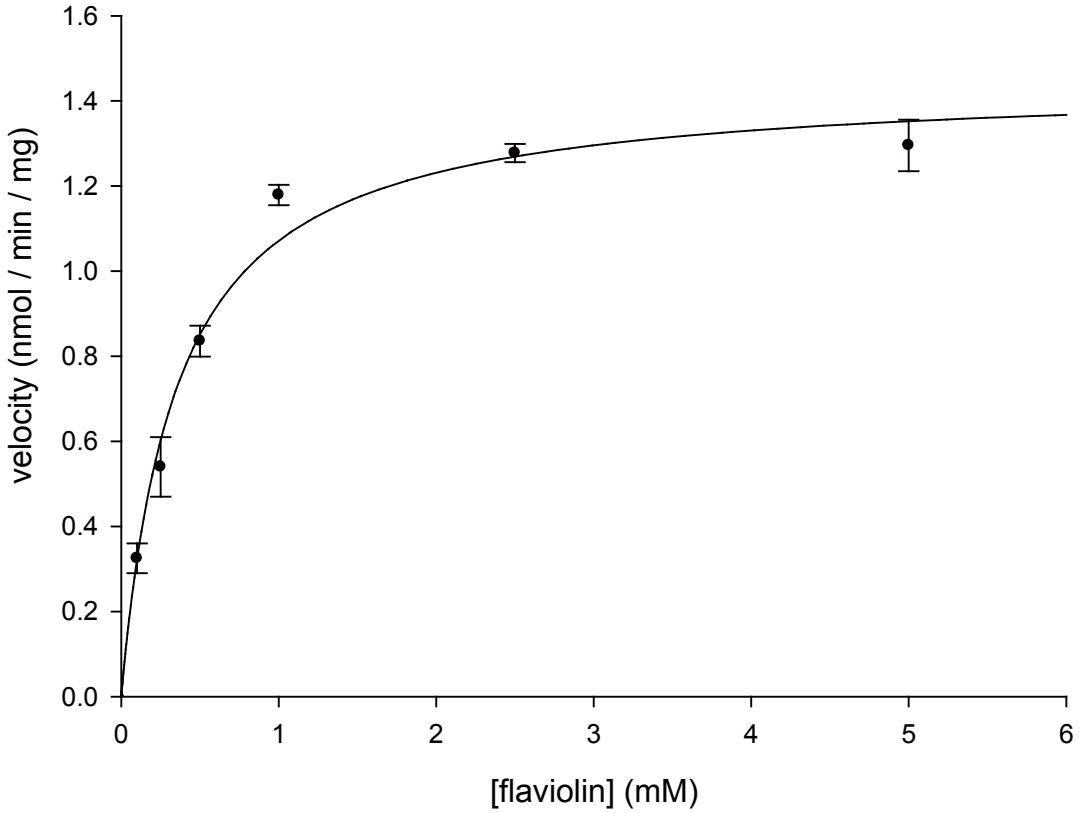

1 / velocity (nmol / min / mg)$^{-1}$
0.02
0.04
0.06
-20
-10
0
10
20
30
1 / [GPP] (mM)

Fig. S4


velocity (nmol / min / mg)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
[flaviolin] (mM)

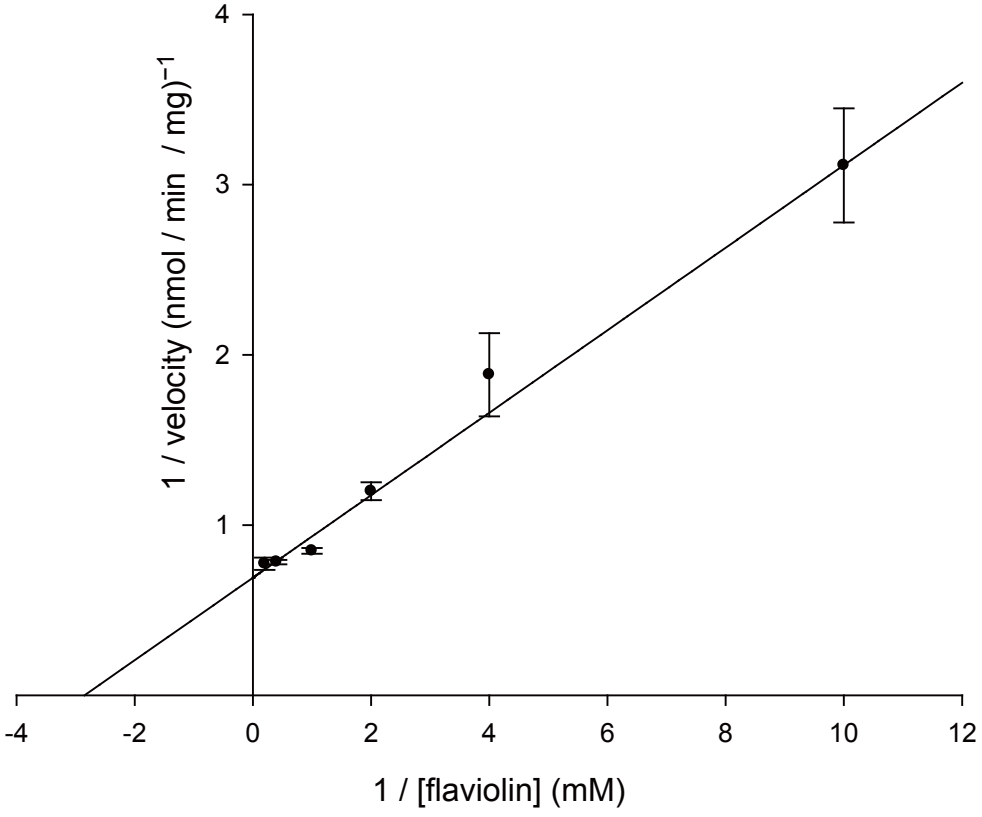
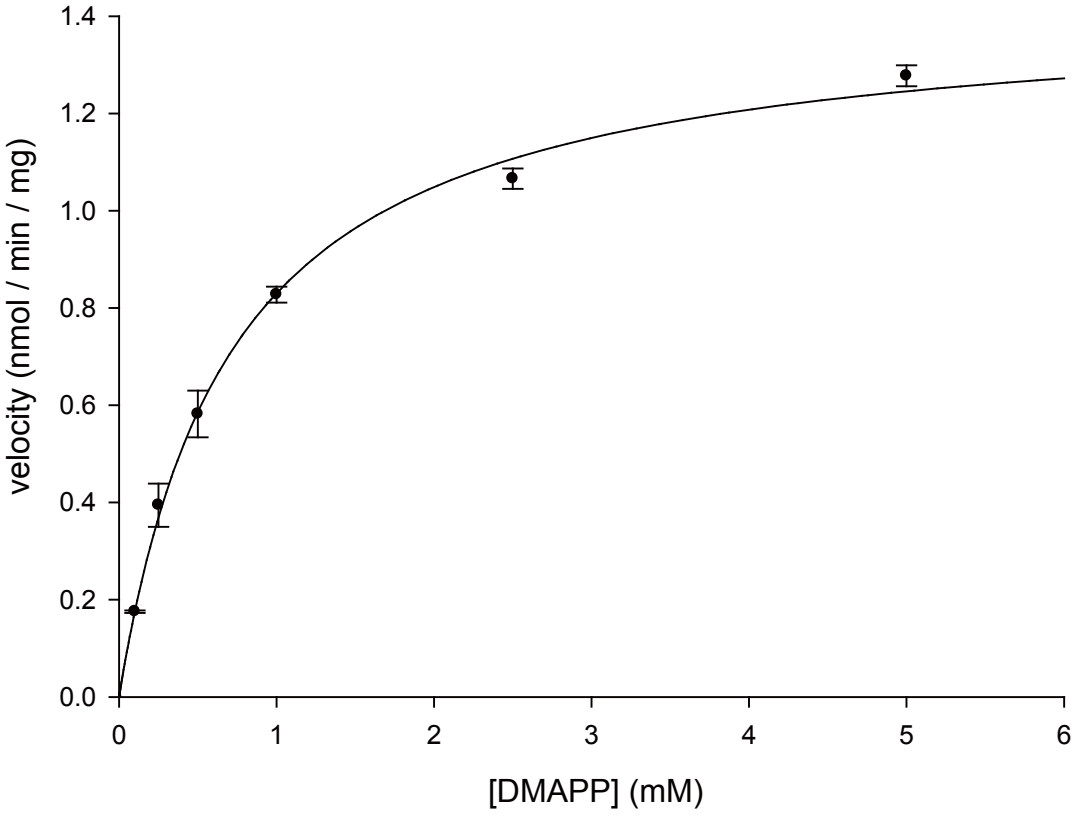

1 / velocity (nmol / min / mg)$^{-1}$
4
3
2
1
-4
-2
0
2
4
6
8
10
12
1 / [flaviolin] (mM)

Fig. S5


velocity (nmol / min / mg)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
[DMAPP] (mM)

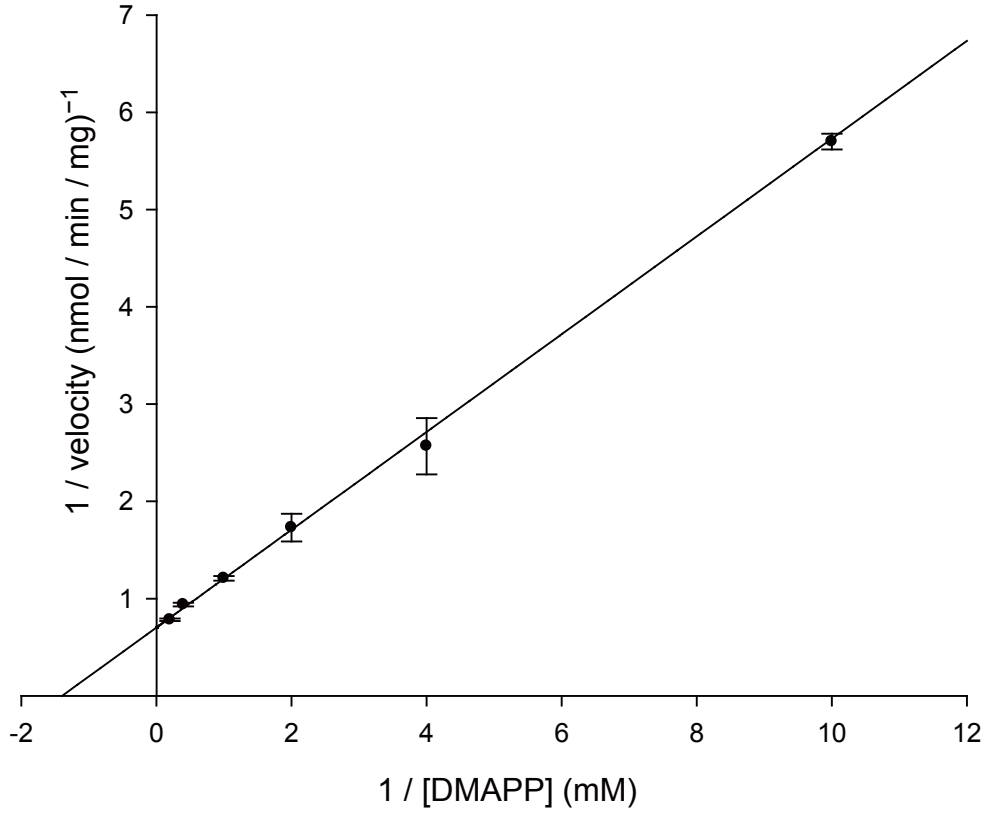
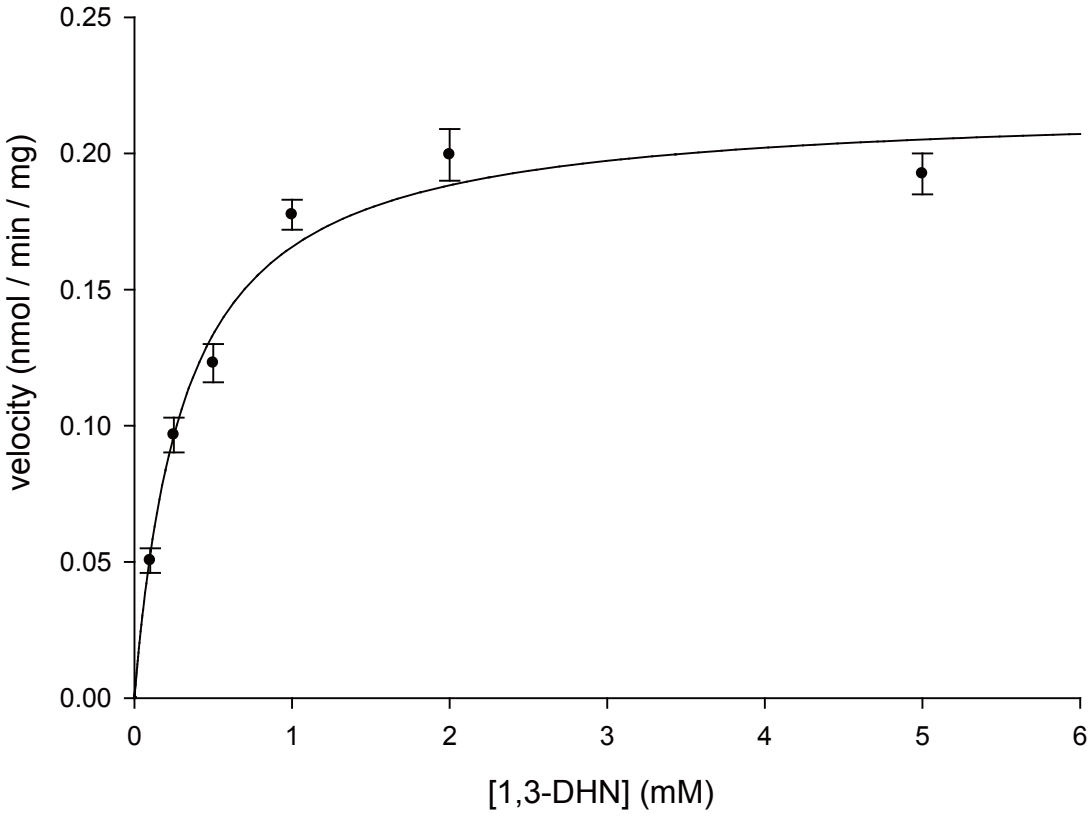

1 / velocity (nmol / min / mg)$^{-1}$
7
6
5
4
3
2
1
-2
0
2
4
6
8
10
12
1 / [DMAPP] (mM)

Fig. S6


velocity (nmol / min / mg)
0.25
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
5
6
[1,3-DHN] (mM)

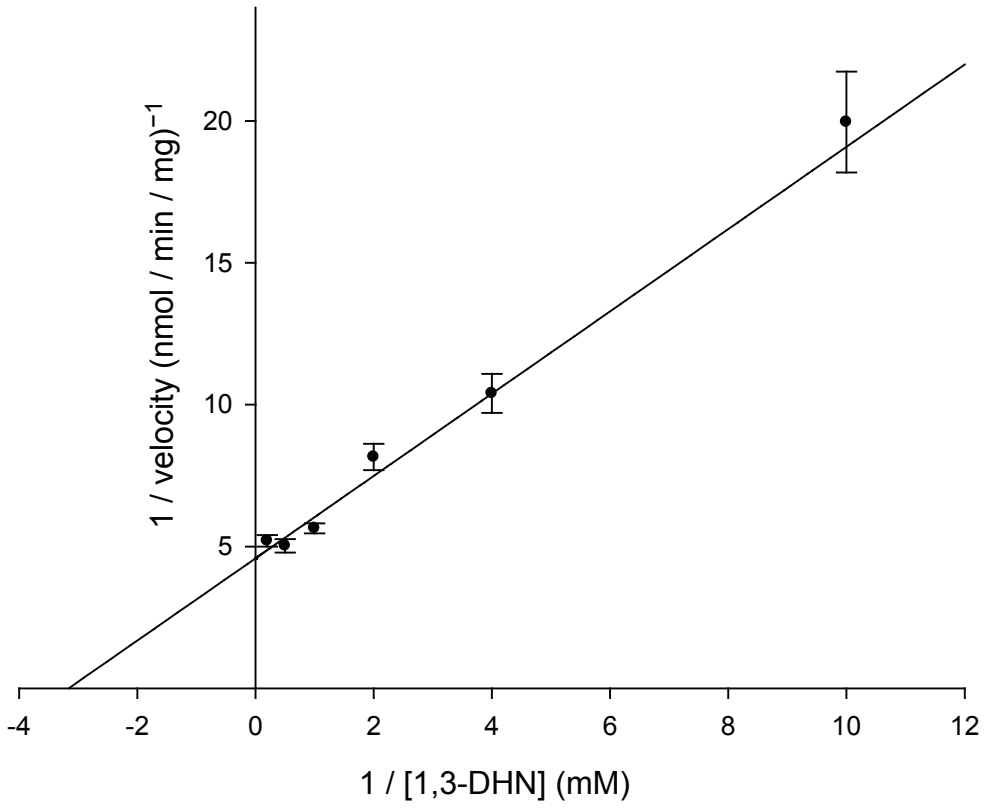
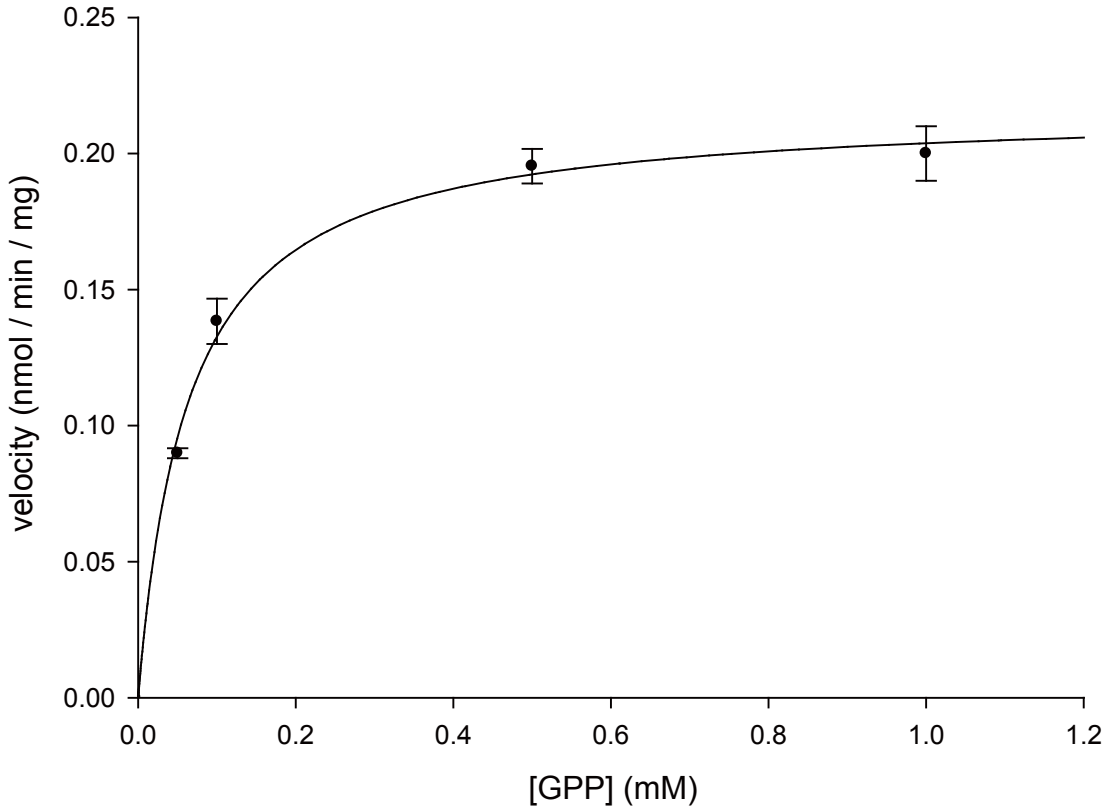

1 / velocity (nmol / min / mg)$^{-1}$
20
15
10
5
-4
-2
0
2
4
6
8
10
12
1 / [1,3-DHN] (mM)

Fig. S7


0.25
0.20
0.15
0.10
0.05
0.00
velocity (nmol / min / mg)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
[GPP] (mM)

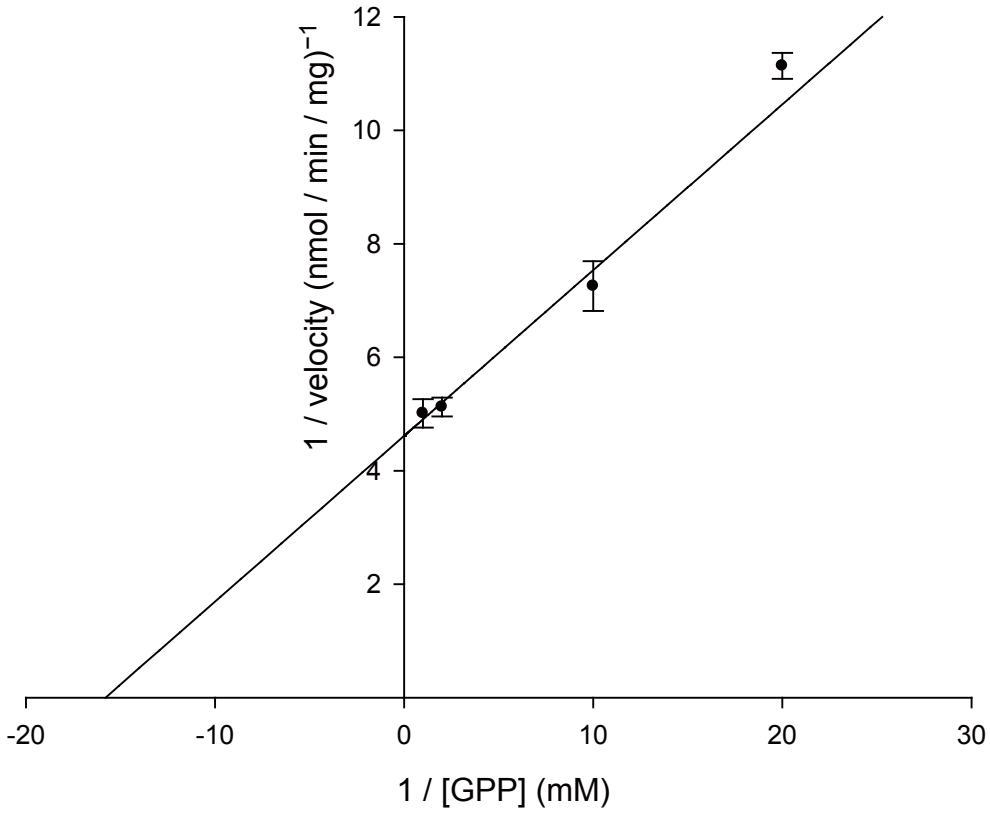

12
10
8
6
4
2
1 / velocity (nmol / min / mg)$^{-1}$
-20
-10
0
10
20
30
1 / [GPP] (mM)

Fig. S8