Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Supporting information

Demethylation of vanillic acid by recombinant LigM in a one-pot cofactor regeneration system

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Figure S1. SDS-PAGE analysis of the purified recombinant LigM and MetE enzymes. Gel was stained with Coomassie blue. (Lane M) Molecular weight marker proteins; (lane 1 and lane 3) crude extract from *E. coli* cells harboring pET24-LigM and pET24-MetE, respectively; (lane 2 and lane 4) purified LigM (20 µg) and MetE (20 µg) after HiTrap Chelating chromatography and dialysis.

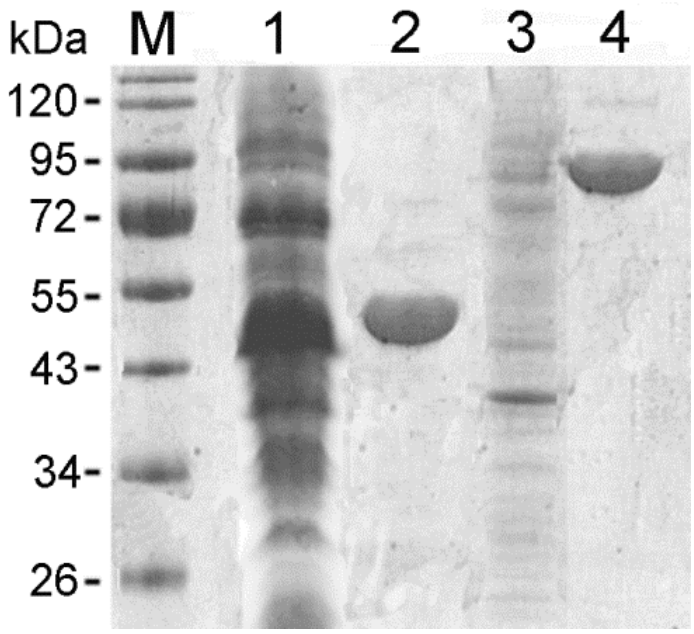

Figure S2. Spectral properties of LigM. a) Protein fluorescence spectrum of LigM (0.1 mg protein/mL) with excitation at 280 nm. b) Near-UV CD spectrum of LigM (0.4 mg protein/mL). c) Far-UV CD spectrum of LigM (0.1 mg protein/mL).

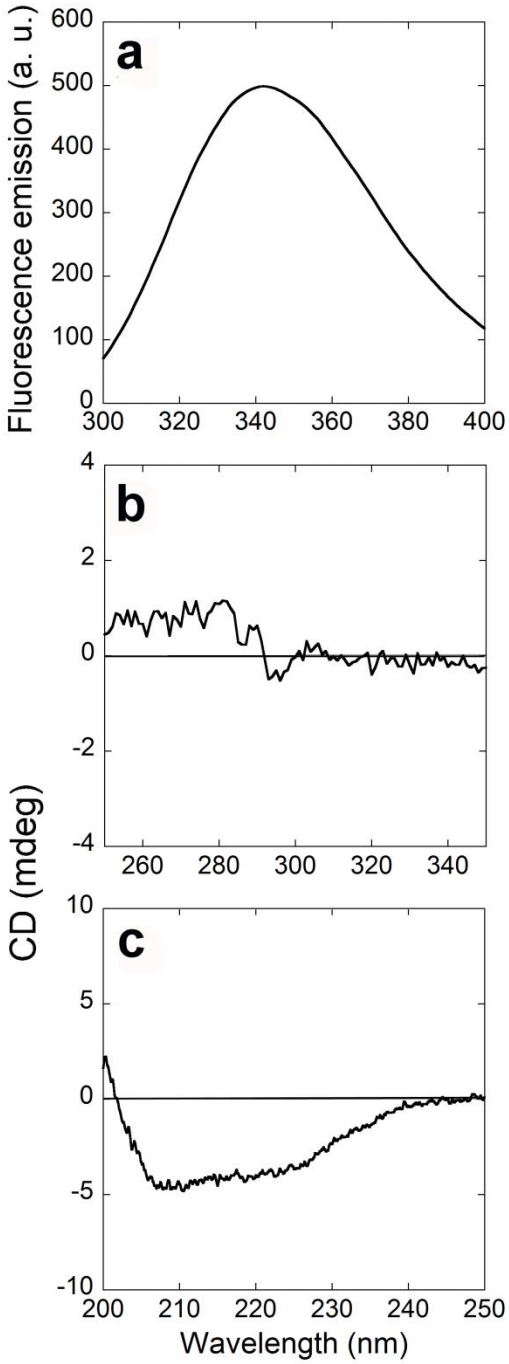

Figure S3. Spectral properties of MetE. a) Protein fluorescence spectrum of MetE (0.1 mg protein/mL), recorded from 300 to 400 nm with excitation at 280 nm. b) Near-UV CD spectrum of MetE (0.4 mg protein/mL). c) Far-UV CD spectrum of MetE (0.1 mg protein/mL). Protein was in 20 mM potassium phosphate, pH 8.0; measurements were performed at 15 °C.

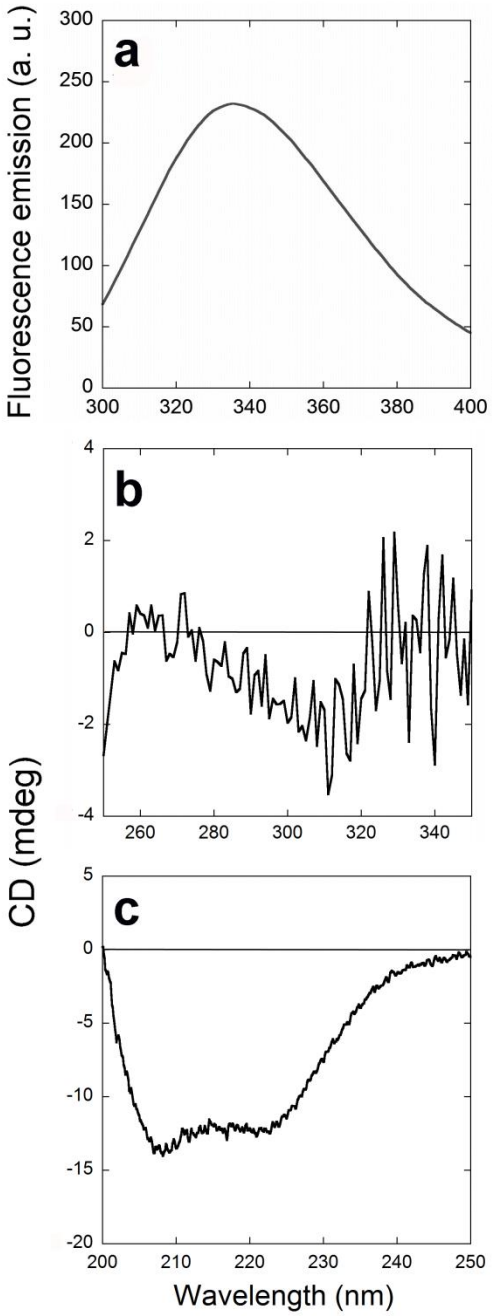

Figure S4. Temperature dependence of different spectroscopic signals for LigM. Spectral signals were monitored continuously during heating from 20 to 80 °C at a heating rate of 0.5 °C/min. Protein (0.1 mg/mL) was in 20 mM potassium phosphate, pH 8.0. (a) Protein fluorescence at 340 nm. (b) CD signal at 220 nm.

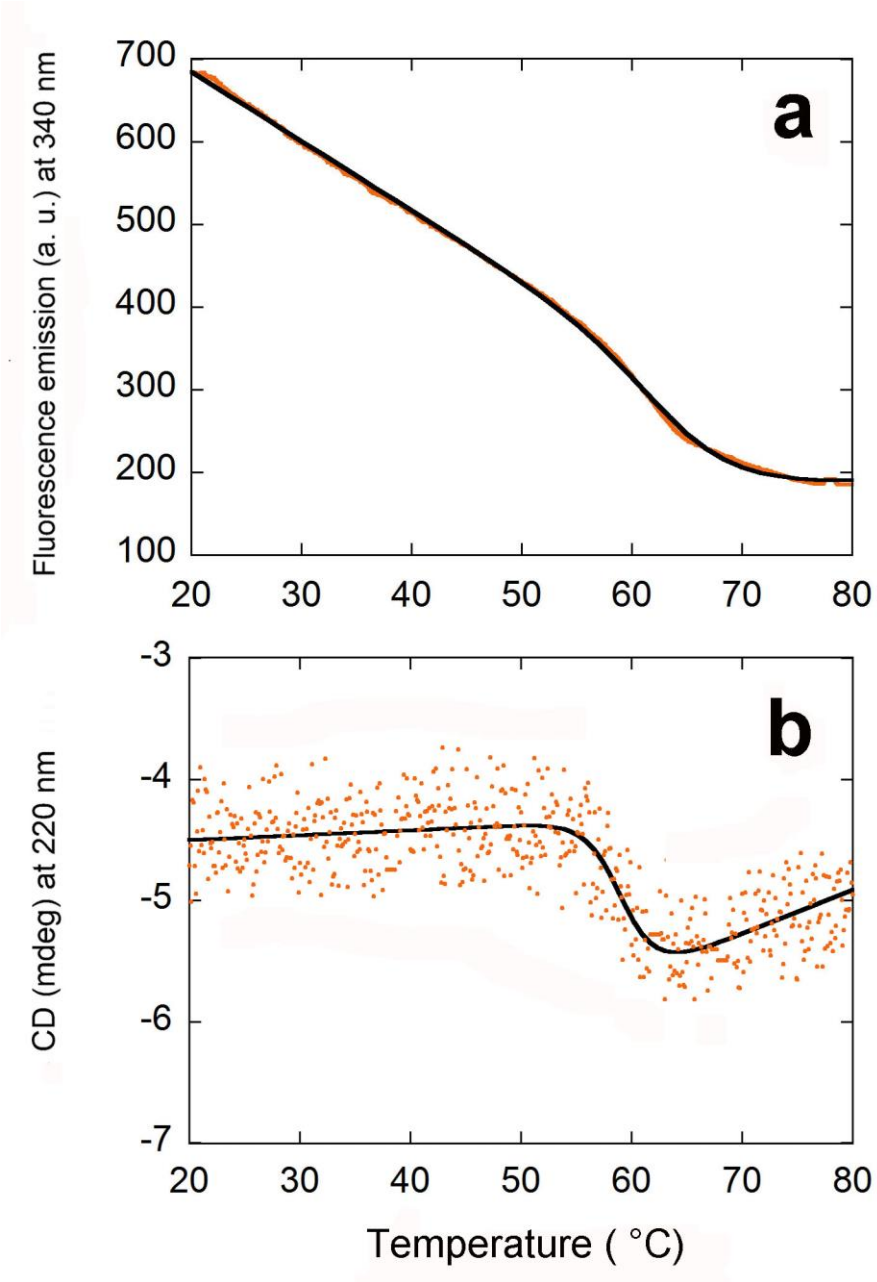

Figure S5. Determination of the dissociation constant for the complex LigM-THF by following the changes in protein fluorescence. 1.8 µM LigM was titrated with increasing amounts of THF, at 15 °C, in 20 mM potassium phosphate pH 8.0.

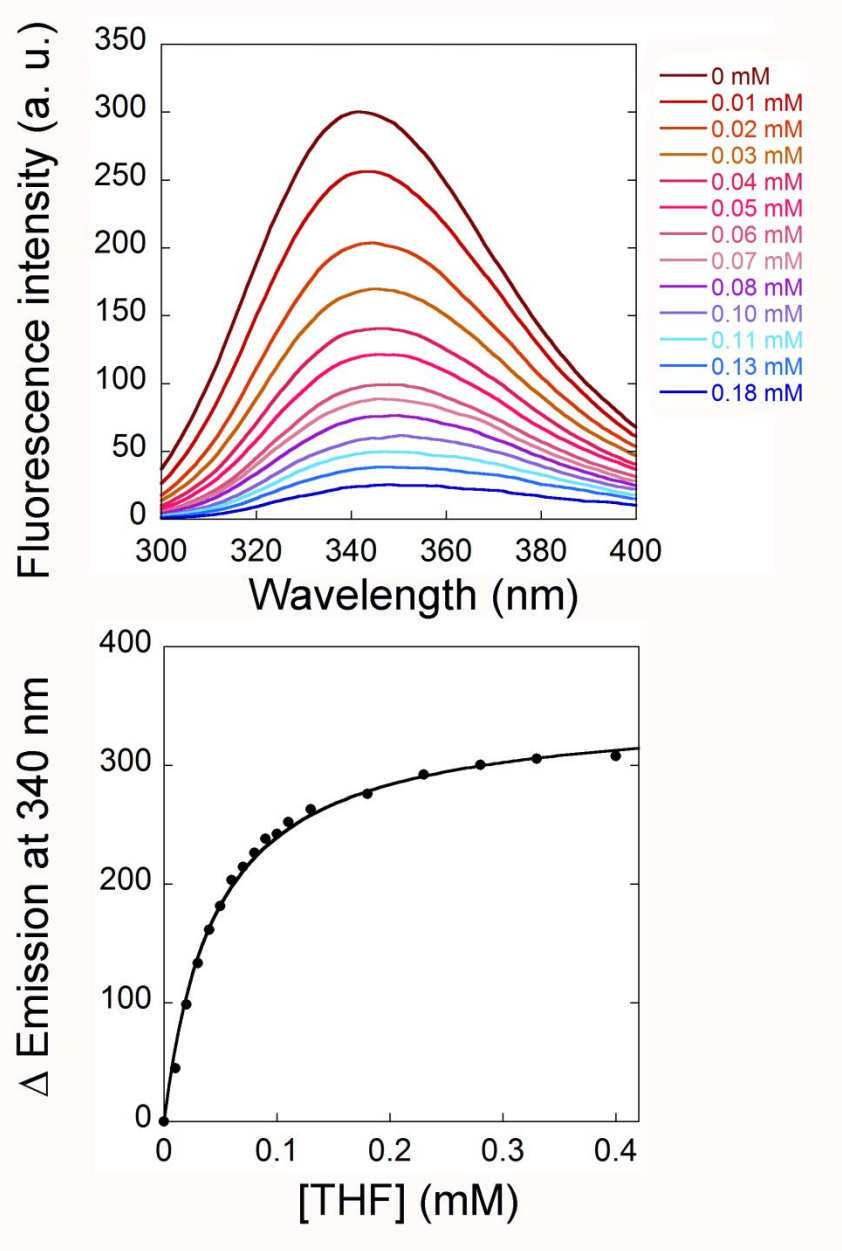

Figure S6. (a) Analysis by negative-ion mode ESI-MS acquisition of reaction mixture of LigM on 0.1 mM vanillic acid after 60 min incubation. (b) Analysis by negative- (top) and positive- (bottom) ion mode ESI-MS acquisition of reaction mixture of LigM and MetE on 1 mM vanillic acid after 240 min incubation. PCA and vanillic acid appear as peak $[M-H]^-$ = 152.8 and $[M-H]^-$ = 166.8, respectively. Methionine and 5-methyl-THF appear as peak $[2M]^+$ = 297.9 and $[M+H]^+$ = 460.8, respectively.

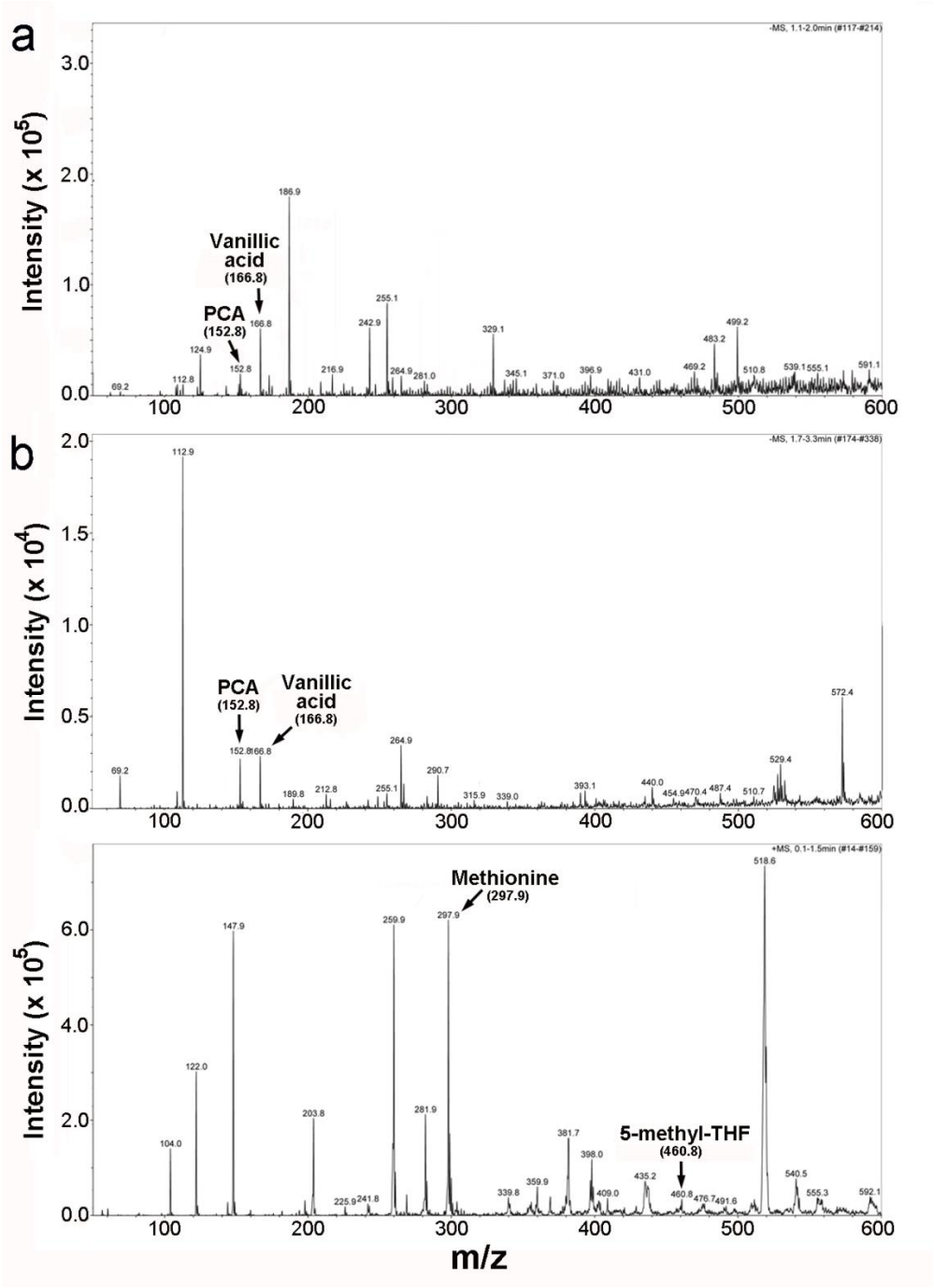

Figure S7. Docking analyses. a-e) Results of docking of vanillic acid, vanillin, ferulic acid, syringic acid and sinapinic acid into the LigM active site with tetrahydrofolate (THF) bound in the active site. All different substrate conformations obtained by using the Autodock Vina program are depicted in gray. In all cases, substrate orientation possessing the lowest docked energy is colored by atom type. See also Figure 1 and Table S1.

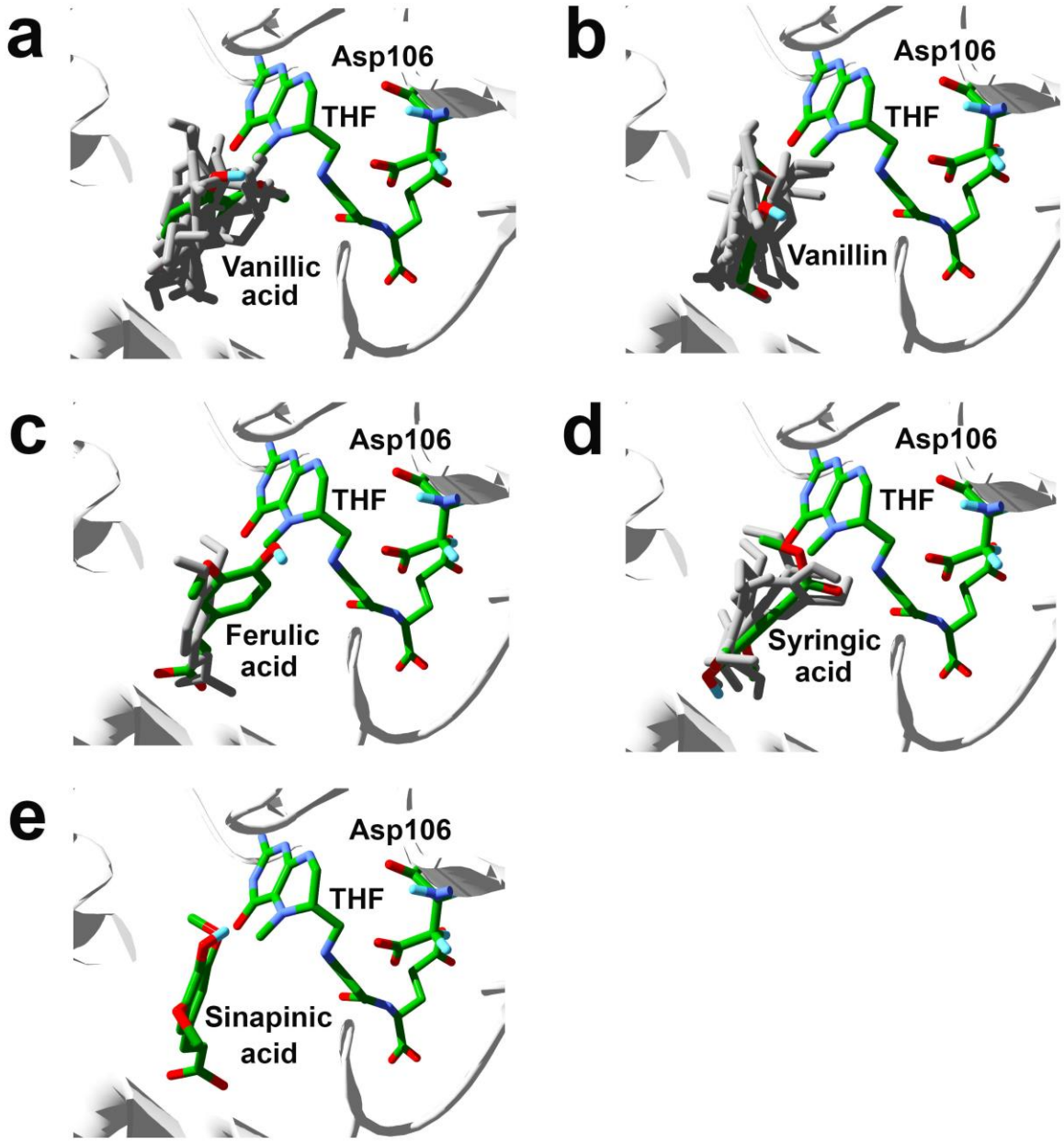

Figure S8. HPLC chromatograms of 5 mM vanillic acid conversion in the presence of 0.1 mM THF prior (continuous line) and 480 min after adding LigM and MetE (dashed line), at 30 °C and pH 8.0. The peak * at 3.8 min contains the THF/5-methyl-THF cofactor.

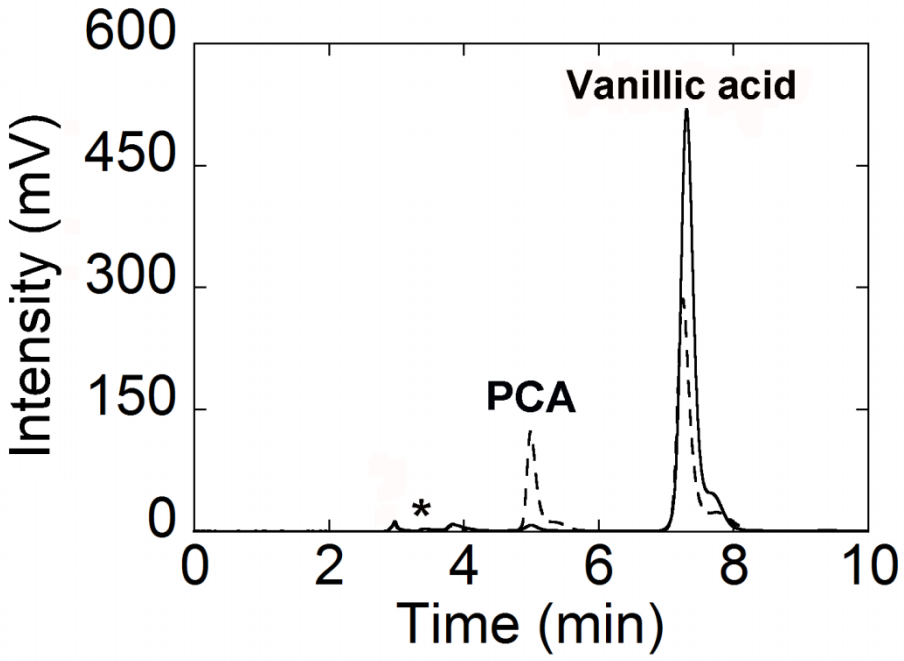

Figure S9. Time course of 0.3 mM vanillic acid conversion by LigM as analysed by HPLC analysis.

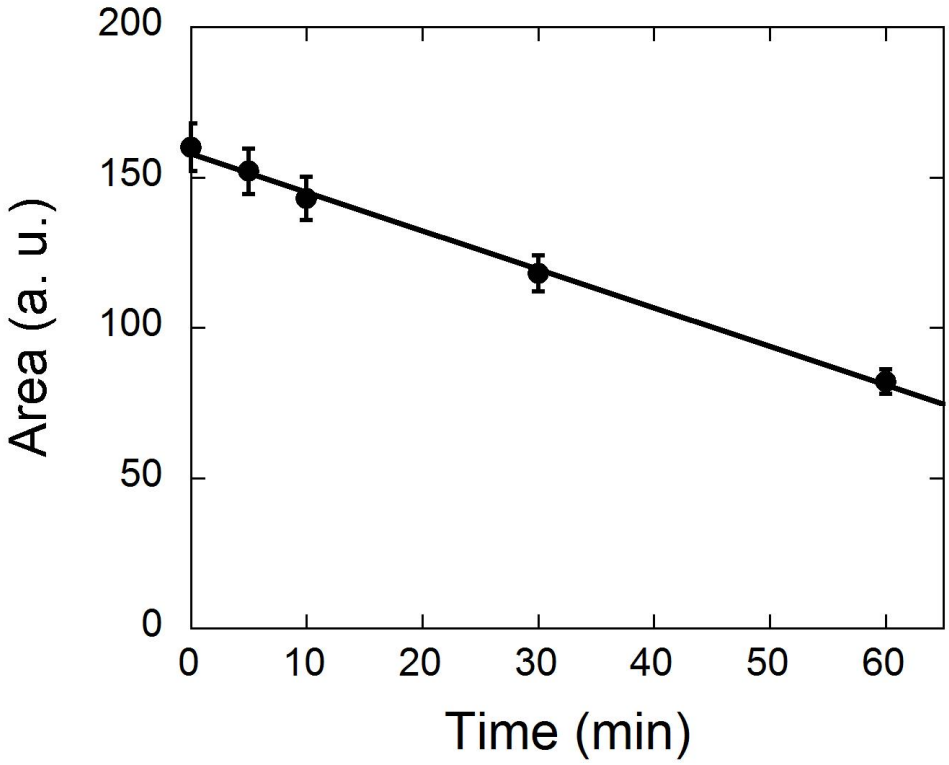

Figure S10. HPLC calibration curves for (a) vanillic acid, (b) protocatechuic acid (PCA), (c) 2,3,4-trimethoxybenzoic acid, and (d) ferulic acid.

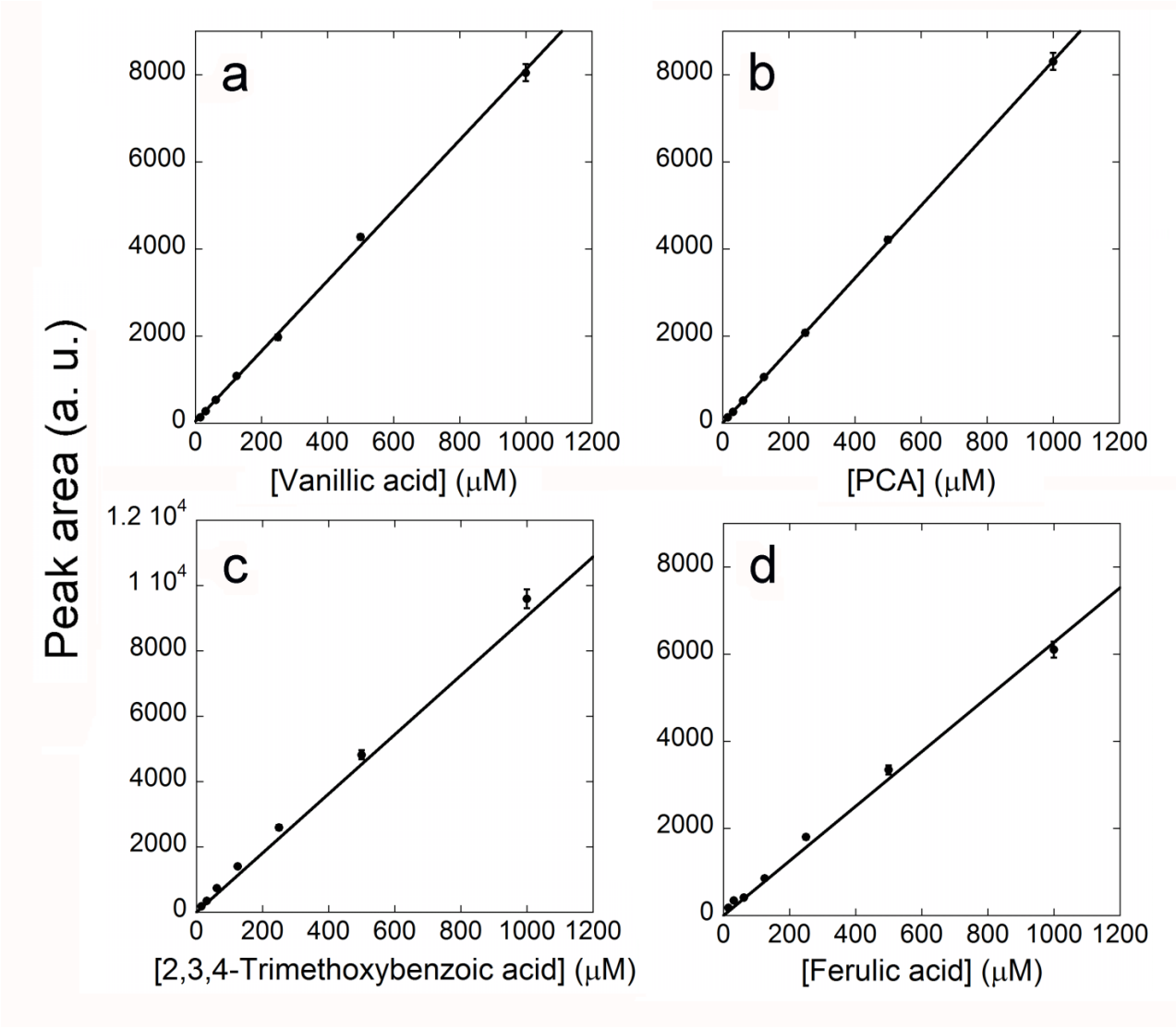

Table S1. Docking results of different compounds to LigM model structure as determined by AutoDock Vina software. Only the results showing a different energy have been reported.

Substrate	Binding mode	Binding energy (kcal/mol)
Vanillic acid	1	-4.4
	2	-4.2
	3	-3.8
	4	-3.8
	5	-3.5
	6	-3.2
	7	-2.9
	8	-2.9
	9	-2.9
Vanillin	1	-4.9
	2	-4.8
	3	-4.8
	4	-4.5
	5	-4.3
	6	-4.2
	7	-4.2
	8	-3.5
	9	-3.4
Ferulic acid	1	-5.1
	2	-4.9
Syringic acid	1	-3.8
	2	-3.5
	3	-3.2
	4	-1.1
Sinapinic acid	1	-4.1