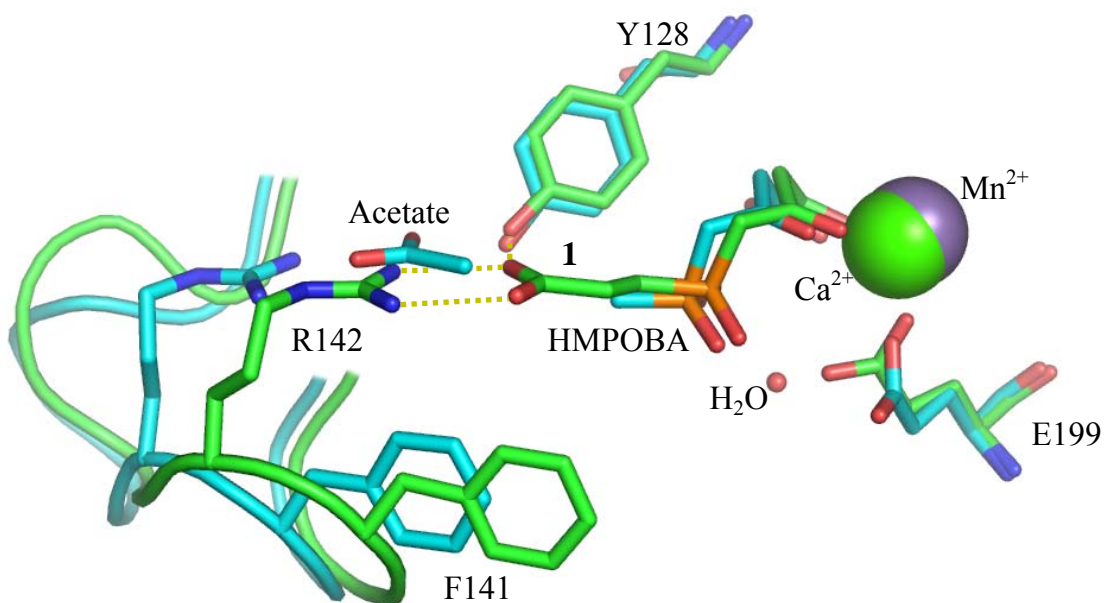


SUPPORTING INFORMATION

Supplementary Figure 1. Overlay outlining significant differences in ligand binding between FAH.1 (green) and the previously determined FAH.HMPOBA (cyan) structure (1HYO). Ribbons represent residues 139-148. Nitrogen, oxygen and phosphorus are colored blue, red, and orange respectively. The catalytic Ca^{2+} of FAH.HMPOBA and Mn^{2+} of FAH.1 are colored green and grey respectively. The water molecule (red) was found only in FAH.HMPOBA. Hydrogen bonding interactions between R142, Y128 and **1** are shown in yellow.



Supplementary Table 1. Relative Activity of GST-hFAH during dialysis¹ at 4° C.

	Day 1	Day 2	Day 3	Day 6	Day 9	Mn(OAc) ₂ ³
No metal	1.00	0.57	0.41	0.23	0.19	0.67
Chelate cocktail ²	1.00	0.50	0.23	0.09	0.05	1.34
Ca(OAc) ₂	1.00	0.64	0.35	0.16	0.11	
Mg(OAc) ₂	1.00	0.65	0.41	0.27	0.22	
Mn(OAc) ₂	1.00	0.98	0.83	0.83	0.78	

¹Dialysis solutions contained 1 mM metal acetate in 20 mM Tris pH 7.4, 150 mM NaCl.

²Chelate cocktail contained 5 mM EDTA, 5 mM EGTA, 5 mM NTA, 5 mM sodium citrate, 20 mM Tris pH 7.4, and 150 mM NaCl.

³ Mn(OAc)₂ was added to the dialysis solutions to “rescue” activity: 1 mM in no metal treatment; and 10 mM to the chelate cocktail treated protein sample (the increased amount being necessary to overcome residual chelating agents present in the solution).

General Methods:

GST-FAH samples were equilibrated using dialysis tubing with an 8,000 molecular weight cutoff against 500 ml of 20 mM TrisHCl, 0.15 M NaCl, pH 7.4 containing 1 mM divalent cations with stirring for 14 to 16 hours at 4 C. Dialyzed samples were concentrated to approximately 10 mg/mL prior to activity determination.

Supplementary Table 2. Ligand H-bond distances from oxygen atoms of **1** to active site residues for individual amino acid chains of the FAH homodimer.

Residue		Compound 1	Chain A (Å)	Chain B (Å)
Arg 142	NH1	O4	2.78	2.83
	NH2	O2	2.95	2.80
Tyr128	OH	O4	2.49	4.52
Tyr128	OH	O2	4.74	2.60
Gln240	Nε2	O6	2.89	2.85
Arg237	NH1	O6	2.81	2.85
Lys253	Nζ	O6	2.89	2.92
His133	Nε2	O7	2.59	2.54
Tyr159	OH	O5	3.21	3.16
Lys253	Nζ	O5	2.88	2.87
Thr350	Oγ1	O1	2.90	2.86
	N	O3	2.91	2.87
Mn ²⁺		O5	2.20	2.20
		O1	2.09	2.11