## Supporting Information

## pH-Rate Profiles Establish that Polyketide Synthase Dehydratase Domains Utilize a Single-Base Mechanism

Xinqiang Xie and David E. Cane\*

Department of Chemistry, Box H, Brown University, Box H, Providence, Rhode Island

02912-9108, United States

\*To whom correspondence should be addressed: <u>david\_cane@brown.edu</u>

## TABLE OF CONTENTS

Supplementary Figures	
Figure S1. LC-MS analysis of the incubation of FosDH1 with (E)-2-butenoyl-SNAC (4).	S3
Figure S2. Initial velocity of FosDH1-catalyzed hydration and dehydration.	S4
Figure S3. FosDH1 activity in sodium phosphate buffer (pH 7.2).	S5
Figure S4. FosDH1-catalyzed dehydration of (3 <i>R</i> )-3-hydroxybutyryl-SNAC (3) pH 5.0 – 9.2.	S6
Figure S5. pH dependence of FosDH1 (Fit of Replicate 2 to Eq. 2 and Eq. 3).	S7
Figure S6. pH dependence of FosDH1 – Fit of Replicates 1 and 2 to Logistic Eq. 4.	S8

Supplementary Table Table S1.

S9



**Figure S1.** LC-MS analysis of the incubation of FosDH1 with (*E*)-2-butenoyl-SNAC (**4**). A1. (*E*)-2butenoyl-SNAC (**4**) standard; A2. MS of retention time between 1 min and 10 min from A1; B1. (3*R*)-3hydroxybutyryl-SNAC (**3**) standard; B2. MS of retention time between 1 min and 10 min from B1; C1. incubation of (*E*)-2-butenoyl-SNAC (**4**) with 1.25  $\mu$ M FosDH1 for 5 min at room temperature; C2. MS of retention time between 1 min and 10 min from C1.



**Figure S2.** Initial velocity of FosDH1-catalyzed hydration and dehydration. **(A)** Progress curve of FosDH1-catalyzed hydration of (*E*)-2-butenoyl-SNAC (**4**) at varying enzyme concentrations (1.25, 2.5 and 5.0  $\mu$ M); **(A1)** Plot of initial velocity *vs.* enzyme concentration using **4** as substrate; **(B)** Progress curve of FosDH1-catalyzed dehydration of (3*R*)-3-hydroxybutyryl-SNAC (**3**) at varying enzyme concentrations (1.25, 2.5 and 5.0  $\mu$ M); **(B1)** Plot of initial velocity *vs.* enzyme concentration using **3** as substrate.



**Figure S3.** FosDH1 activity in sodium phosphate buffer (pH 7.2). Top plot: hydration of (*E*)-2-butenoyl-SNAC (**4**). Bottom plot: dehydration of (3R)-3-hydroxybutyryl-SNAC (**3**).



**Figure S4.** FosDH1-catalyzed dehydration of (3R)-3-hydroxybutyryl-SNAC (**3**) at pH 5.0 – 9.2 (no activity was detected at pH 3.0 or 4.0). A. Replicate 1. B. Replicate 2



Figure S5. pH dependence of FosDH1 (Fit of Replicate 2 to Eq. 2 and Eq. 3).

Alternative Statistical Fit of pH Dependence of FosDH1. Since Eq. 2 and Eq 3 for the pH dependence of  $log(k_{cat})$  and  $log(k_{cat}/K_m)$  do not permit inclusion of rate values of 0, an alternative statistical analysis of the pH dependence of  $k_{cat}$  and  $k_{cat}/K_m$  over the full range pH 3.0 – 9.2 was also carried out using the Logistic expression of Eq. 4, as suggested by one of the Referees (Figure S6):

 $Y = A2 + (A1-A2)/(1 + (x/x_0)^p)$  (Eq 4)

where

y...k<sub>cat</sub> or k<sub>cat</sub>/K<sub>m</sub>

- A1 minimum value of  $k_{cat}$  or  $k_{cat}/K_m$
- A2 maximum value of  $k_{cat}$  or  $k_{cat}/K_m$
- x pH
- $x_0$  sigmoid point or inflection point = pKa

p steepness of the curve;  $log_{10} p$  gives the Hill coefficient



**Figure S6.** pH dependence  $k_{cat}$  of FosDH1 – Fit of Replicates 1 and 2 to Logistic Eq. 4.

**Table S1.** Steady-state kinetic parameters for FosDH-1-catalyzed dehydration of (3*R*)-3-hydroxybutyryl-SNAC (**3**) from pH 3.0 to 9.2.

рΗ	Replicate 1			Replicate 2		
	k <sub>cat</sub> (min <sup>-1</sup> )	K <sub>m</sub> (mM)	<i>k</i> <sub>cat</sub> / <i>K</i> <sub>m</sub> (M <sup>-1</sup> min <sup>-1</sup> )	k <sub>cat</sub> (min <sup>-1</sup> )	<i>K</i> <sub>m</sub> (mM)	k <sub>cat</sub> /K <sub>m</sub> (M⁻¹min⁻¹)
3.0	0		0	0		0
4.0	0		0	0		0
5.0	2±0.2	29±5	69	1.3±01	19±3	68
6.0	7.4±0.5	30±4	247	9.5±2	44±18	216
7.0	20±3	51±12	385	18±2	43±8	419
8.0	26±1	66±3	394	24±1	60±3	400
9.2	23±1.5	57±6	404	26±2	64±10	406