(Reference numbers in the Supporting Information correspond to those used in the text.)

Kinetic Method

The kinetic method is the same as that used previously.[5d,8,9] A four-syringe stopped-flow kinetic unit (Applied Photophysics, SX-18) was used, and reactions were studied at 4 °C. Solutions containing CYP119 (20 μM) and MCPBA (20 μM) were mixed in the first push of the unit, and the mixture was aged for 100 ms to allow accumulation of Compound I. The Compound I solution was then mixed with a solution containing excess substrate, and kinetics were followed at 416 nm (λ_{max}) of the resting enzyme. First-order reactions were observed. Figure S1 shows a time-resolved UV-visible spectrum from reaction of CYP119 Compound I in the absence of substrate, which is similar to those previously reported.[5d,8] Figure S1 was obtained with a diode array detector. The predominant changes in absorbance are decay at ca. 360 nm (λ_{max} for Compound I) and growth at 416 nm (λ_{max} for the ferric protein). In the kinetic studies, monochromatic light at 416 nm was followed with a photomultiplier tube to achieve better signal-to-noise than possible with the diode array detector. Figure S2 shows an example for a set of studies of reaction with palmitic acid at pH 7.2.

The observed first-order rate constants (k_{obs}) obtained in the presence of substrate were corrected for reaction of Compound I in the absence of substrate by subtracting the background reaction (k_0). Plots of the data versus the concentration of substrate showed curvature indicating saturation kinetics, except in the case of pH 7.4 reactions where the change in rate constant with substrate concentration was so great that the data appeared linear (see Figure 1 of text). The data was solved for saturation kinetics according the reaction model discussed below, and the results are reported in Table 1 in the text.

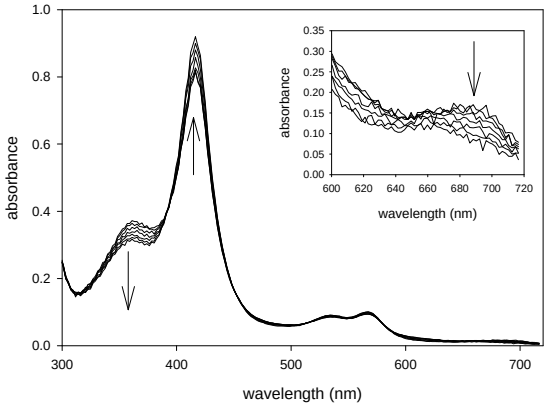


Figure S1. Time-resolved spectrum from stopped-flow mixing of CYP119 with MCPBA in 100 mM phosphate buffer (pH 7.0) at 4 °C recorded at 40 ms, 100 ms, 200 ms, 300 ms, 400 ms, 500 ms, and 600 ms after mixing. The inset shows an expansion of the range 600 nm to 720 nm.

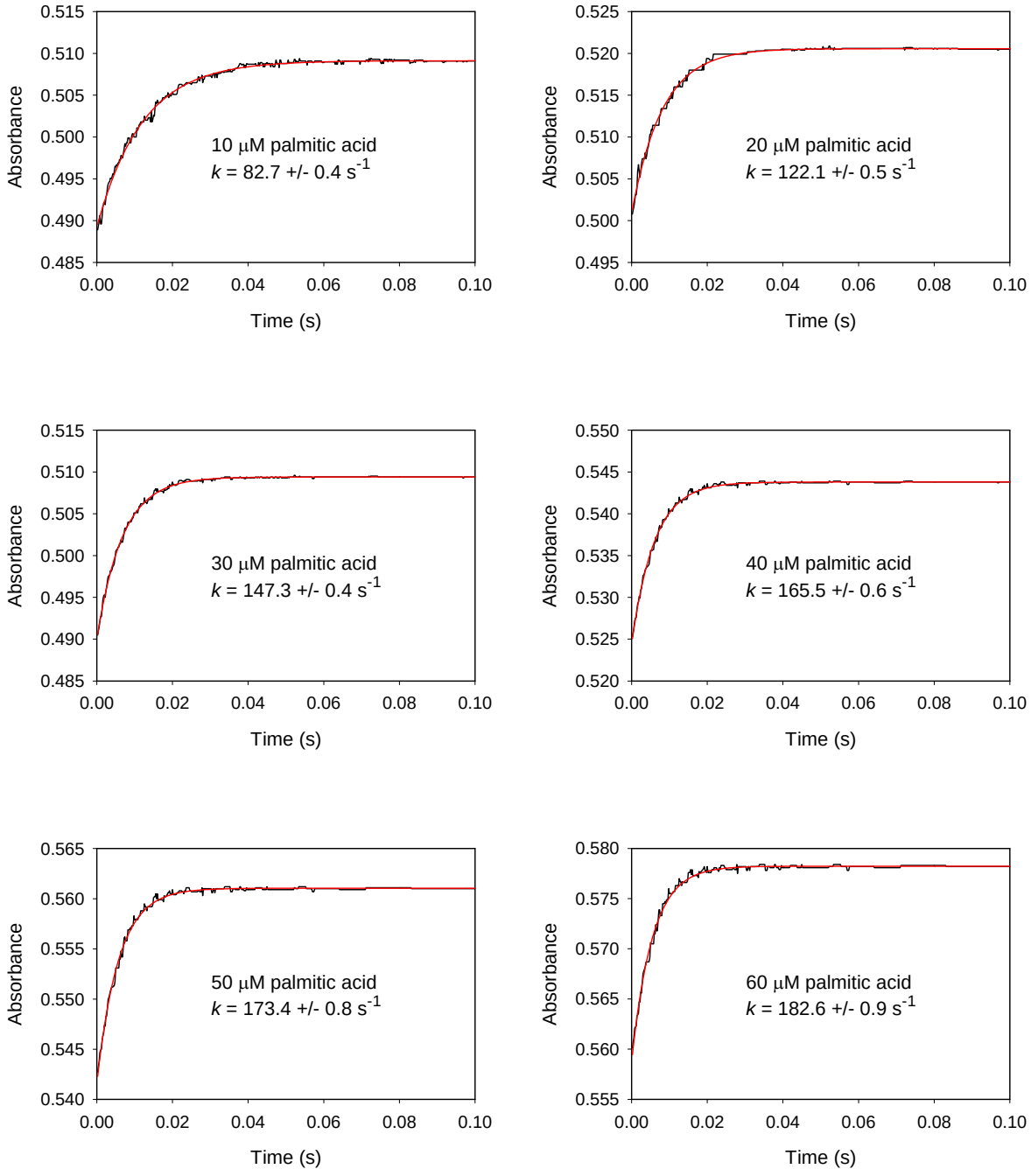
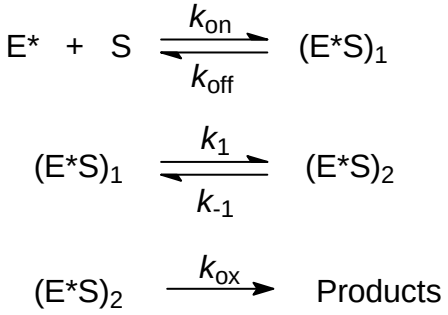


Figure S2. Kinetic traces at λ = 416 nm for reactions of CYP119 Compound I with palmitic acid at 4 °C in 100 mM phosphate buffer at pH 7.2. The data is shown in black, and the fit for a first-order growth is shown in red. The concentrations of substrate and first-order rate constants are listed on the plots.

Reaction Models

The reaction model employed is shown in Scheme 1. It is similar to but not exactly the same as Michaelis-Menten kinetics. The difference is that we are studying single turnover reactions, and the rate of release of first-formed product from the enzyme cannot appear in the rate law. We define an apparent binding constant as $K_{bind} = k_{on}/(k_{off} + k_{exp})$. This term is an association constant that is related to the Michaelis constant K_M, which is given as a dissociation constant; i.e. $K_{bind} = 1/K_M$ if the product dissociation rate is not in the rate law. The experimental rate constant k_{exp} is a first-order reaction rate constant; this term is the equivalent of V_{max} in the Michaelis-Menten derivation if experiments are conducted at constant enzyme concentrations.

Scheme S1



For reactions at pH ≤ 7.3, saturation kinetics were observed (Figure 1 of text), which requires that the activated enzyme forms a complex reversibly with substrate and that the complex reacts with a first-order rate constant. Saturation kinetics are solved by Eq S1, where k_{obs} is the observed rate constant, k_0 is the rate constant for reaction in the absence of substrate, K_{bind} is the pseudo-equilibrium constant for formation of the complex, k_{exp} is the first-order rate constant for reaction of the complex, and [Sub] is the concentration of substrate. The values for K_{bind} and k_{exp} are listed in Table 1 of the text.

$$(k_{obs} - k_0) = (K_{bind}\, k_{exp}\, [Sub])/(K_{bind}\, [Sub] + 1) \qquad (S1)$$

At pH = 7.4, the rate of change of the rate constants was so steep that we were unable to observe curvature in the plots of $(k_{obs} - k_0)$ versus [Sub]. Accordingly, the pH 7.4 data was solved for apparent second order rate constants, and the results are listed in the text.

For dodecanoic acid and hexadecanoic acid, the perdeuterated isotopomers reacted with apparent binding constants K_{bind} and experimental rate constants k_{exp} that were the same as those obtained for the non-deuterated isotopomers. This requires that the C-H oxidation reaction was not the slow step in the process, and the reaction model shown in Scheme 1 applies. For these acids, $k_{ox} > k_{-1}$, and the experimental rate constant is $k_{exp} = k_1$. For octanoic acid, partial KIEs were observed indicating that $k_{ox} \approx k_{-1}$. The full KIEs found in intramolecular studies of partially deuterated dodecanoic acid and hexadecanoic acid are reported in the text, and one can assume that the same intramolecular KIEs would be found for octanoic acid.

Mixed Third-Order and Fourth-Order Kinetics

The k_{exp} values were plotted against pH in a log—log format that gives the kinetic order of the rate constant on hydroxide concentration as the slope of the line (Figure 2 in the text). These plots displayed good linearity, and the highly precise data for palmitic acid gave a slope of

3.5 ± 0.1. In the simplest model for mixed kinetic order, a third-order and a fourth-order reaction compete.

If competing third-order and fourth-order reactions in hydroxide concentration have equal rate constants at pH 7.0, then the relative rate constants for the two reactions and the total rate constant are listed in Table S1. The total rate constant found under these conditions will appear to have kinetic order of 3.5 over the range pH 6.8 to 7.3 as shown by the log—log plot for k_{TOT} in Figure S3, where the slope is 3.5. We normalized the experimental rate constants for reactions of palmitic acid with CYP119 Compound I at pH 7.0 and plotted that data in Figure S3; the experimental data nearly superimposes on the simulated data.

Table S1. Relative Rate Constants for Competing Third- and Fourth-Order Reactions.

pH	k_3	k_4	k_{TOT}
6.8	0.25	0.16	0.41
6.9	0.50	0.40	0.90
7.0	1.00	1.00	2.00
7.1	2.00	2.51	4.51
7.2	3.98	6.31	10.29
7.3	7.94	15.85	23.79

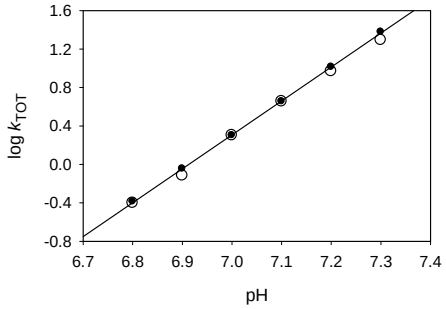


Figure S3. Log--log plot of total rate constants from Table S1 (solid circles); the slope of the line is 3.52. Kinetic data for hexadecanoic acid normalized to $k = 2.00$ at pH 7.0 is also plotted (open circles).

References

(The reference numbers used in the Supporting Information are the same as those in the text.)

5d. J. Rittle, M. T. Green, *Science* **2010**, *330*, 933-937.

8. Z. Su, X. Chen, J. H. Horner, M. Newcomb, *Chem. Eur. J.* **2012**, *18*, 2472-2476.

9. X. H. Chen, Z. Su, J. H. Horner, M. Newcomb, *Org. Biomolec. Chem.* **2011**, *9*, 7427-7433.