Appendix D: Derivation of apparent correlation between ^{18}O-KIE and $K_m(O_2)$

To reconcile the apparent correlation between ^{18}O-KIEs and $K_m(O_2)$ as shown in Fig. 4, we consider a simple two-step enzymatic reaction involving a reversible O_2 binding step and an irreversible reaction step converting enzyme-bound O_2 into products (either H_2O_2 or hydroxylated organic substrate) as shown in Eq. D1,

$$O_2 \underset{k_2}{\overset{k_1}{\leftrightarrow}} \text{E-}O_2 \overset{k_3}{\rightarrow} \text{P} \qquad \text{(D1)}$$

where E-O_2 is the enzyme-bound O_2, P represents the reaction products, and k_1, k_2, and k_3 are elementary reaction rate constants of the forward and backward reactions. In this case, the measured ^{18}O-KIE is related to the intrinsic equilibrium and kinetic isotope effects of the two elementary steps through the forward commitment to catalysis, k_3/k_2, as shown in Eq. D2 (Cleland 2005),

$$^{18}\text{O-}\text{KIE}_{\text{measured}} = \frac{\text{EIE}_1\text{KIE}_3 + {}^{k_3}/_{k_2}\text{KIE}_1}{1 + {}^{k_3}/_{k_2}} \qquad \text{(D2)}$$

where EIE_1 is the equilibrium isotope effect of the O_2 binding step and KIE_1 and KIE_3 are the kinetic isotope effects of the O_2 binding and reaction steps associated with rates k_1 and k_3, respectively. From Eq. D2, two extreme cases can be derived. If O_2 binding alone is rate-limiting ($k_3 >> k_2$), the measured ^{18}O-KIE will approximate KIE_1. If the second reaction step is rate-limiting ($k_3 << k_2$), the measured ^{18}O-KIE will approximate the product of EIE_1 and KIE_3. When we start with the latter case, which has a small k_3/k_2, and increase the forward commitment gradually, the measured ^{18}O-KIE will slowly decrease assuming KIE_1 is smaller than EIE_1KIE_3. For such a reaction, plotting measured ^{18}O-KIEs vs. k_3/k_2 will yield a similar (apparently linear) trend as shown in Fig. 4 as long as the commitment factor (k_3/k_2) is below 1. As shown in Eq. D3, k_3/k_2 can be related to $K_m(O_2)$, if we consider $K_m(O_2)$ to be $(k_3 + k_2)/k_1$ and K_d, the dissociation constant of O_2, to be k_2/k_1. The trend observed in Fig. 4 can thus be explained if K_d varies much less than $K_m(O_2)$ for this set of enzymes.

$$\frac{k_3}{k_2} = \frac{k_3}{k_2} + \frac{k_2}{k_2} - 1 = \frac{k_3 + k_2}{k_2} - 1 = \frac{K_m(O_2)}{K_d} - 1 \qquad \text{(D3)}$$