SUPPLEMENTAL ONLINE FIGURES

Supplemental Figure 1. Sensitivity analysis to determine parameter ranges: When other kinetic rates are kept at default values, kinetic off-rates corresponding to the minimum HIF1α half-lives of 5 and 8 min respectively are: $k_{cat,H\alpha}$ = 0.098-0.164 min^{-1} (i), $k_{off,Fe2}$ = 0.019-36 min^{-1} (ii), $k_{off,DG}$ = 0.044-10.8 min^{-1} (iii), $k_{off,O2}$ = 0.044-10.8 min^{-1} (iv), and $k_{off,AS}$ = 0.0036-3.6 min^{-1} (v). Values of $k_{off,DG}$ and $k_{off,O2}$ above 10.8 min^{-1} yield the same response curve as that for 10.8 min^{-1}. (vi) The off-rate for unhydroxylated HIF1α corresponding to a minimum HIF1α half-life of 5 min is $k_{off,H\alpha}$ = 0.7 min^{-1}; the sensitivity to this parameter is low, with all response curves intersecting beyond 6 min.

Supplemental Figure 2. Comparison of initial reaction rate to experiments was used to narrow the kinetic parameter ranges. (i) $k_{cat,H\alpha}$ = 0.098-0.164 min^{-1} corresponds to minimum HIF1α half-lives of 5 and 8 min respectively, and this range compares well with the specific activity from (Tuckerman et al., 2004). This $k_{cat,H\alpha}$ range was used in calculations of subsequent kinetic parameters. (ii) From the minimum range of 0.019-36 min^{-1} calculated for $k_{off,Fe2}$ from HIF1α half-lives, $k_{off,Fe2}$ = 36 min^{-1} corresponds best to the experimentally-determined specific activity. (iii) $k_{off,DG}$ = 10.8 min^{-1} is the analogous value for 2-oxoglutarate unbinding to PHD2. (iv) For O_2, this value is $k_{off,O2}$ = 10.8 min^{-1} and for ascorbate, $k_{off,AS}$ = 3.6 min^{-1} (v). $k_{off,H\alpha}$ = 0.7 min^{-1} was the best value within the range estimated from half-life comparisons, that correlates with the specific activity data shown (vi).

Supplemental Figure 3. Sensitivity of HIF1α hydroxylation to concentrations of reactants. The upper curve in both graphs corresponds to initial concentrations of unhydroxylated $[HIF1\alpha]_0$ = 1 μM; $[PHD2]_0$ = 1 μM; and $[Fe2^+]_0$ = 50 μM, as frequently used by in vitro experiments. (i) Decreasing $[Fe^{2+}]$ results in a large reduction of hydroxylation. In vivo, total cellular Fe^{2+} concentrations vary from 20-200 μM (Arredondo et al., 1997; Hirsila et al., 2005). The fraction that is freely available for binding to PHD2 depends on cell type; in cultured insect cells used for HIF1α experiments, it has been estimated as 0.3-0.8

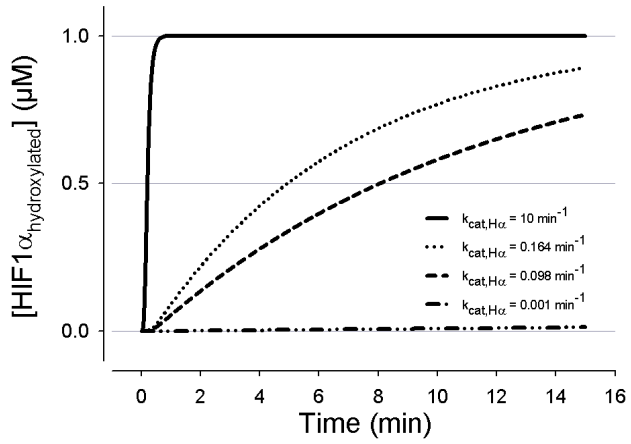
μM (without addition of Fe^{2+} to the culture) (Esposito et al., 2002). (ii) Decreasing $[PHD2]_0$ also results in a steep reduction of hydroxylation. In vivo levels of PHD2, if they can be approximated by in vitro cell extracts, are on the order of 4 nM (Tuckerman et al., 2004). Compared to Fe^{2+} and PHD2, ascorbate has minimal effects on each cycle of the hydroxylation reaction; see Figure 2.

Supplemental Figure 4. Using the newly reported K_m value of 0.03 μM for binding of iron to PHD2 (Hirsila et al., 2005), the model predicts a maximum specific activity of 411 mol/mol/min {calculated from the slope of the tangent to the curve in (i)}, during the first six minutes of the hydroxylation reaction. This is 10-fold greater than predicted by (Hirsila et al., 2005), where minimum PHD2 specific activity is estimated as 40-50 mol/mol/min. An exhaustive search for parameter sets using $K_{m,Fe2}$ = 0.03 μM found no binding on and off-rates that represented well both HIF1α half-lives and PHD2 specific activity reported in literature. The closest match, yielding a HIF1α half-life of 8 min (ii), was found using the rates shown in (i).

Supplemental Figure 5. Calculations of half-lives (i, iii) and PHD2 specific activity (ii, iv) from the model, with different Michaelis-Menten constant K_m values. Sensitivity analysis was performed for all kinetic parameters found from experiments, and comparisons were made to experiments on HIF1α half-life (Berra et al., 2001) and PHD2 specific activity (Tuckerman et al., 2004). Examples are shown for $K_{m,Fe2}$ (i,ii) and $K_{m,DG}$ (iii,iv).

Supplemental Figure 6. Relative HIF1α hydroxylated at different O_2 levels after 5 and 20 minutes, for different values of K_m's. K_m is the experimentally determined Michaelis-Menten constant. Examples are shown for iron, $0.025K_{m,Fe2}$ to 2 $K_{m,Fe2}$ (i), and for 2-oxoglutarate, $0.2K_{m,DG}$ to $2K_{m,DG}$ (ii).

SUPPLEMENTAL FIGURE 1



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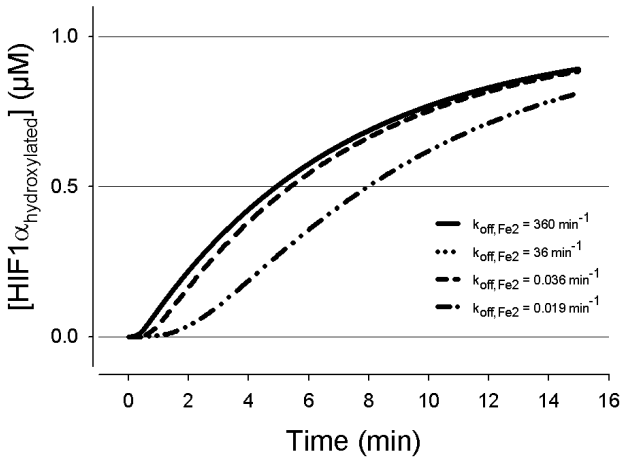

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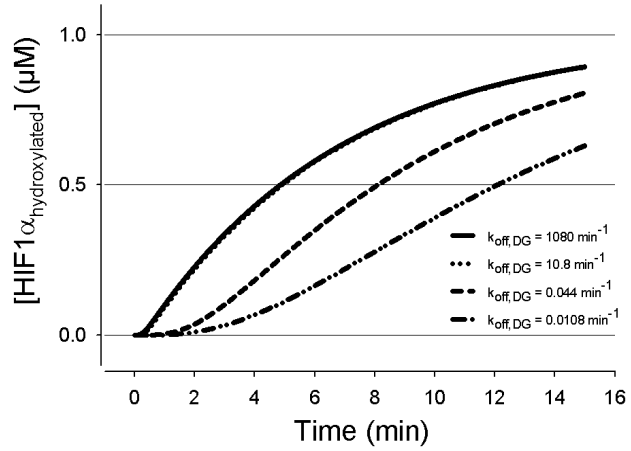

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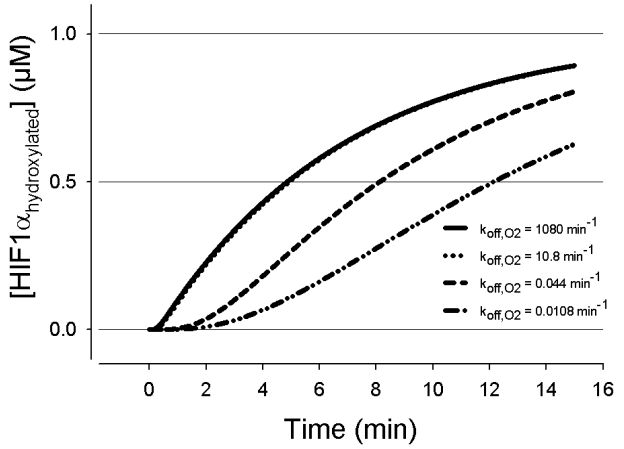

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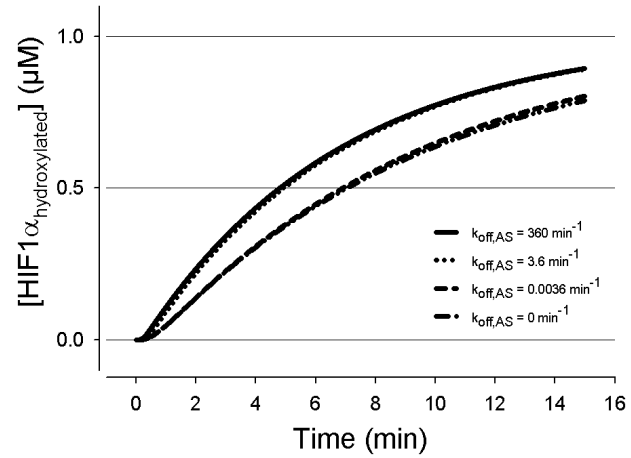

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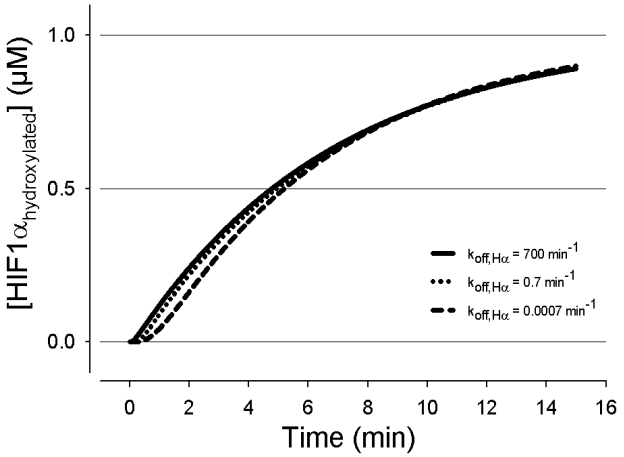

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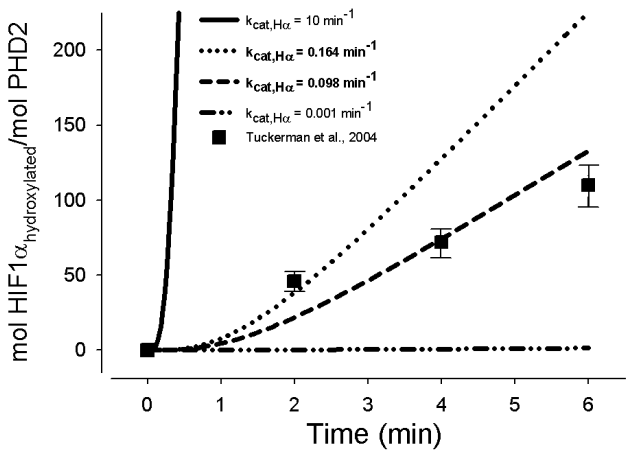

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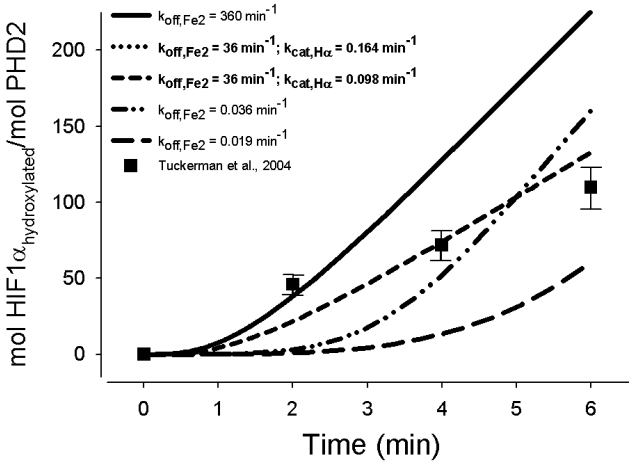

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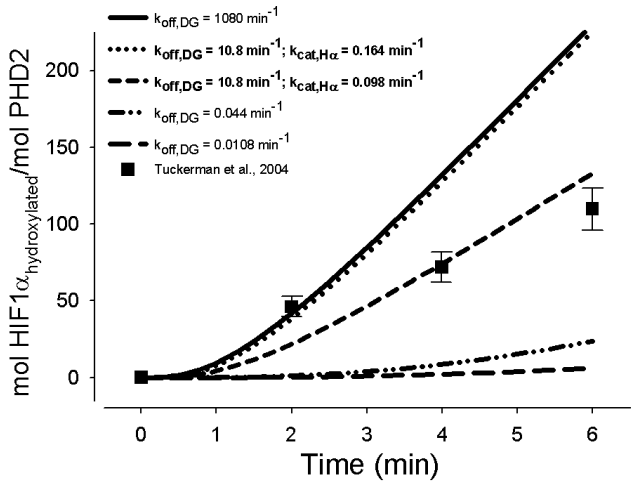

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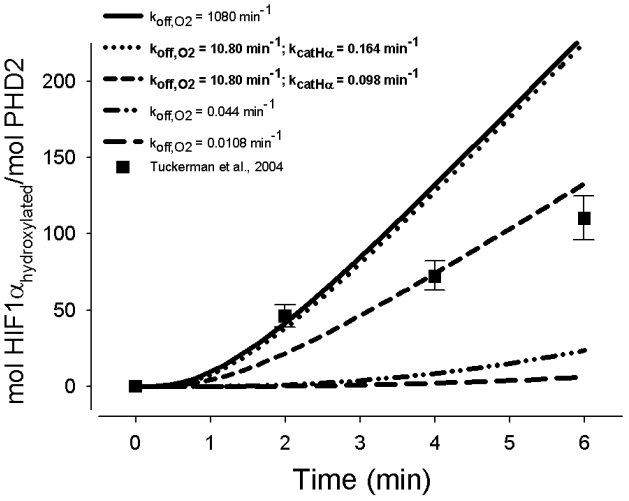

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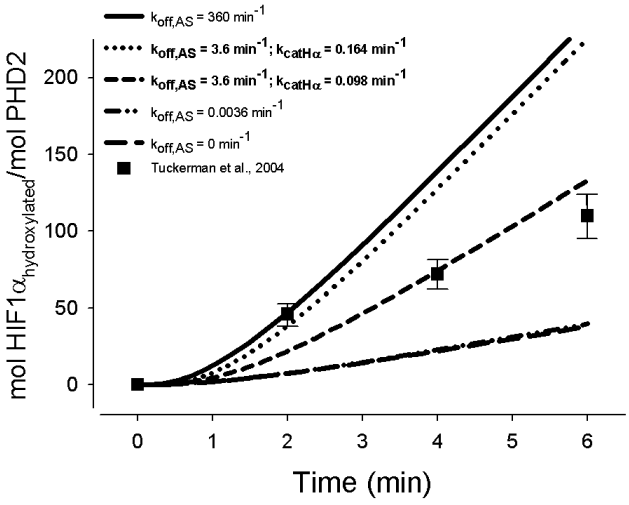

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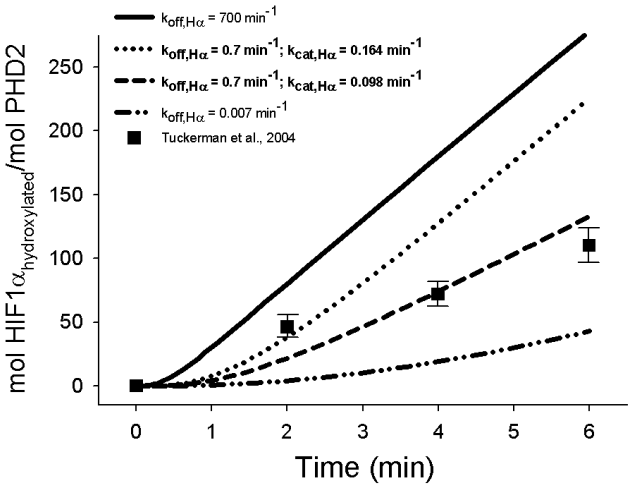

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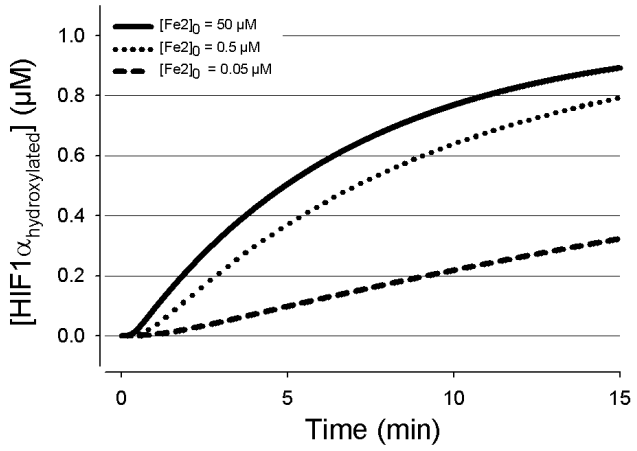

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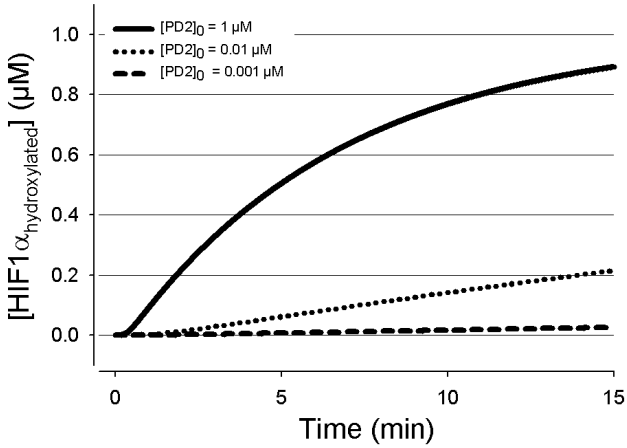

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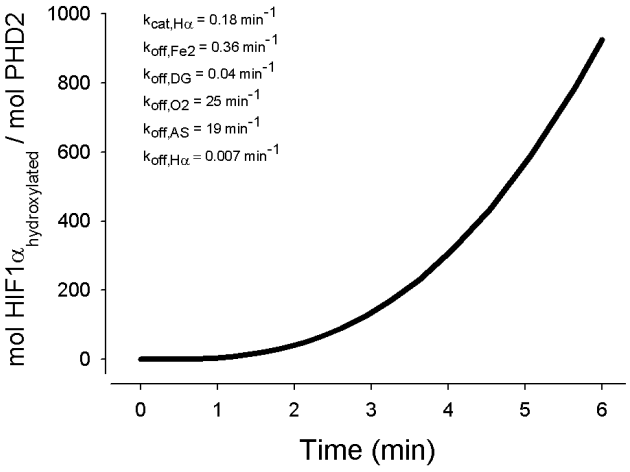

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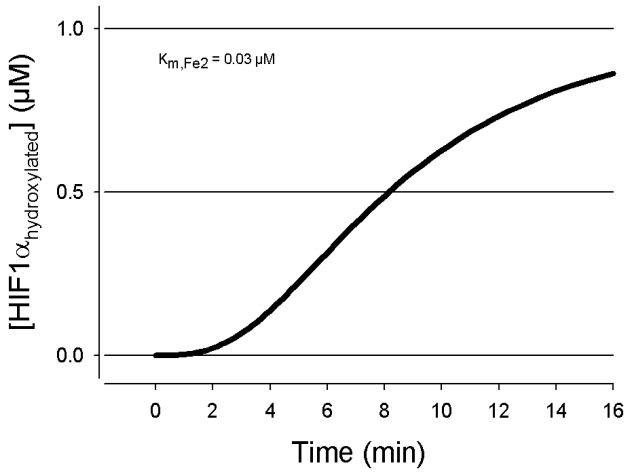

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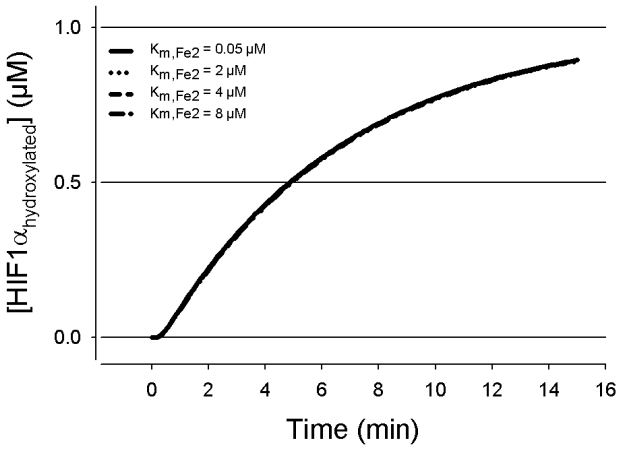

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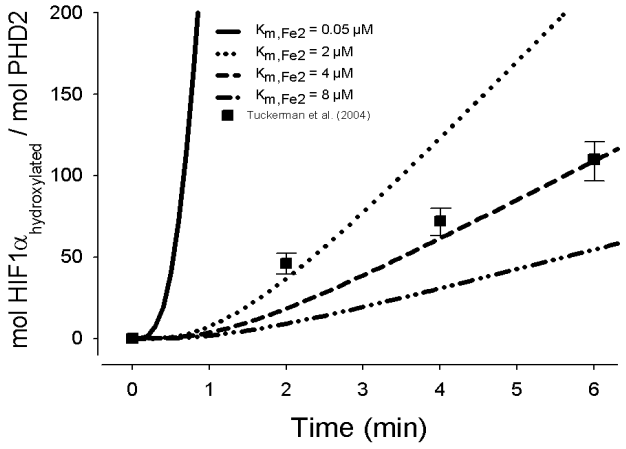

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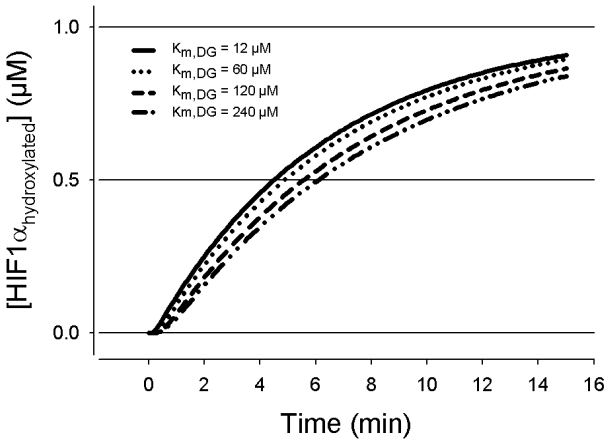

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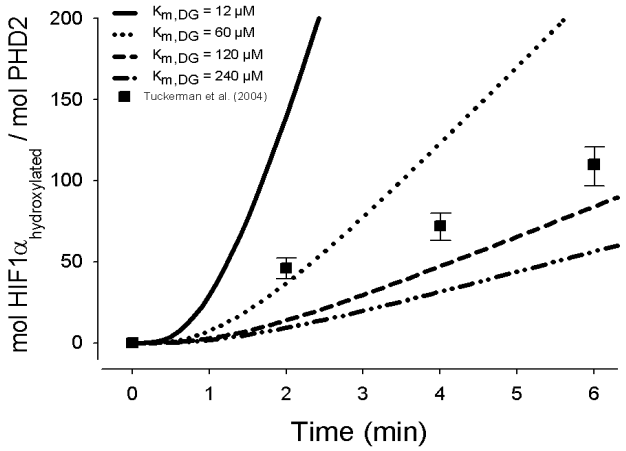

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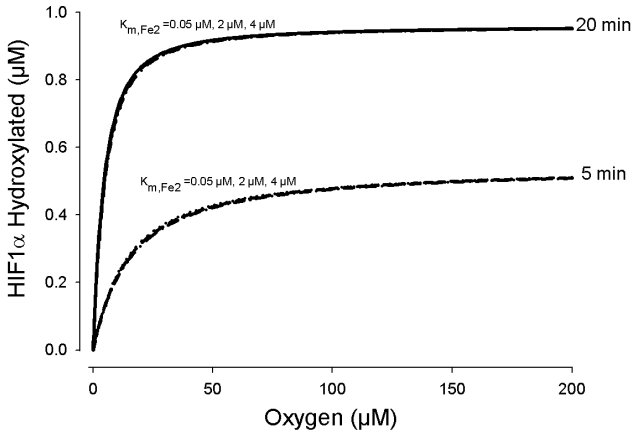

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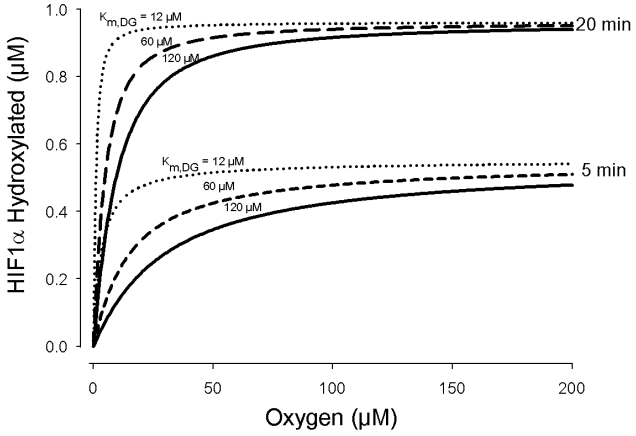

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