1. The hydroxylation rate functions.

Michaelis-Menten kinetics are only strictly valid if the substrate is in excess over the enzyme, and if formation of enzyme-substrate complexes does not significantly decrease the concentration of free substrate [1]. In protein-protein interaction networks, this is not necessarily true. In our case, the concentration of the substrate HIFα can become very low, and cannot be assumed to be in excess over the hydroxylases at all times. Thus, we model the HIFα hydroxylation reactions by taking into account that free HIFα is decreased by complex formation with the hydroxylases. Figure S4 compares the results obtained by this method with a Michaelis-Menten approximation. Section 2 derives the expressions for PHD-dependent ODD-hydroxylation in detail. HIFα CAD-hydroxylation by FIH in the presence of competing ARD proteins (Section 3) as well as ARH-hydroxylation (Section 4) follow the same scheme. All expressions obtained reduce to simple Michaelis-Menten-type kinetics if substrate concentrations are large compared to enzyme concentrations.

2. Derivation of a rate function for HIFα ODD-hydroxylation.

There is strong evidence that oxygen and HIFα can bind to hydroxylases independently of each other [2], which gives rise to the following reaction scheme. $H_p$ indicates all forms of HIFα that can bind to PHD (i.e. are not already bound to PHD). $P_0$ is PHD not bound by either HIFα or oxygen, and $P_1, P_2, P_{12}$ indicate complexes of PHD bound to oxygen, HIFα, or both, respectively.

 Binding of HIFα to PHD has been suggested to be fast compared to the enzyme’s reaction with oxygen [4], and we treat binding reactions B and D as at steady state. In this case,

$$k_{on}P_1H_p = k_{off}P_{12}$$

and (2.1) simplifies to (2.3), where $K_p^p$ is the Michaelis constant of PHD for oxygen. From this equilibrium assumption, we also obtain (2.4) and (2.5), where $K_D^p$ indicates the dissociation constant of the PHD/HIFα complex.

$$\frac{dP_{12}}{dt} = k_{on}(P_2O_2 - K_p^p P_{12})$$

$$P_2 = P_0 \frac{H_p}{K_D^p}$$

$$P_{12} = P_1 \frac{H_p}{K_D^p}$$

The total amount of PHD, $P_{tot}$, is conserved, and with (2.4) and (2.5) given as

$$P_{tot} = P_0 + P_1 + P_2 + P_{12} = (P_0 + P_1) \frac{K_D^p + H_p}{K_D^p}$$

If the productive complex $P_{12}$ is at steady state, (2.3) equals zero, and with (2.3) and (2.4) we obtain

$$P_{12} = P_0 \frac{O_2}{K_M + O_2} \frac{H_p}{K_D^p}$$

Combining (2.7), with (2.5) yields (2.8), which we substitute into (2.6) to obtain (2.9), which, with (2.7) yields the expression for the productive complex (2.10) and thus the hydroxylation (=degradation) rate (2.11).

$$P_0 = P_{tot} \left( \frac{K_D^p}{K_D^p + H_p} \left( \frac{K_M^p}{K_M + O_2} \right) \right)$$

$$P_{12} = P_{tot} \left( \frac{H_p}{K_D^p + H_p} \left( \frac{O_2}{K_D^p + O_2} \right) \right)$$

$$- \frac{dH_{tot}}{dt} = k_p H_{tot} P_{tot} \left( \frac{H_p}{K_D^p + H_p} \left( \frac{O_2}{K_D^p + O_2} \right) \right)$$

In the classical Michaelis-Menten approximation, the amount of substrate bound to enzyme is considered negligible and $H_p$ is replaced by $H_{tot}$, the total amount of HIFα present in the system. Using (2.6), we obtain $H_p$ as an explicit function of $H_{tot}$ from mass conservation:

$$H_{tot} = H_p + P_2 + P_{12} = H_p + P_{tot} \frac{H_p}{K_D^p + H_p}$$

$$H_p + P_2 (K_D^p + P_{tot} - H_{tot}) - K_D^p H_{tot} = 0$$

$$H_p = \frac{1}{2} \left( H_{tot} - K_D^p + \sqrt{(K_D^p + P_{tot} - H_{tot})^2 + 4K_D^p H_{tot}} \right)$$

The given solution is the biologically relevant of the two roots of the quadratic equation. Finally, we rewrite (2.12) to obtain (2.15), which we combine with (2.11) to obtain our final expression for the hydroxylation and thus degradation.

---

**Diagram:**

- **A.** $P_0 + O_2 \rightarrow P_1$
- **B.** $P_0 + H_p \rightarrow P_2$
- **C.** $P_2 + O_2 \rightarrow P_{12}$
- **D.** $P_1 + H_p \rightarrow P_{12}$

Catalysis: $P_{12} \rightarrow P_0 + H_{OH} \rightarrow P_0$

ODD-hydroxylated HIF is very unstable and, as a first approximation, degraded instantaneously [3]. The hydroxylation rate can thus be viewed as a degradation rate and is equal to the catalytic turnover of the productive complex, $P_{12}$, for which we need to derive an expression. The change of $P_{12}$ with time is given by the differential equation (2.1), where $k_{on}$ and $k_{off}$ are the on- and off-rate constants for oxygen binding, and $k_{on}$ and $k_{off}$ for HIFα binding, respectively.

$$\frac{dP_{12}}{dt} = k_{on}P_2O_2 + k_{on}P_1H_p - P_{12}(k_{off} + k_{on} + k_{off})$$

1
rate (2.16). Division by $H_{tot}$ gives the rate function $v_p$ for HIFα hydroxylation by PHD (2.17), which we will use in the system of ODEs.

$$H_F = H_{tot} \frac{K_F^p + H_F}{K_F^p + H_F + P_{tot}}$$

$$\frac{dH_{tot}}{dt} = k_p F_{12} = k_p F_{tot} \left( \frac{H_{tot}}{K_F^p + H_F + P_{tot}} \right) \left( \frac{O_2}{K_M^p + O_2} \right)$$

$$v_p = k_p F_{tot} \left( \frac{1}{K_F^p + H_F + P_{tot}} \right) \left( \frac{O_2}{K_M^p + O_2} \right)$$

It is immediately clear from (2.15) that, for small enzyme concentrations, $H_F \approx H_{tot}$, in this case the rate equation (2.16) becomes a classical Michaelis-Menten-type function (2.18). Expressions of this form are used in Skeleton Models 1 and 2.

$$\frac{dH_{tot}}{dt} = k_p F_{12} = k_p F_{tot} \left( \frac{H_{tot}}{K_F^p + H_F + P_{tot}} \right) \left( \frac{O_2}{K_M^p + O_2} \right)$$

3. Derivation of a rate function for HIFα CAD-hydroxylation in the presence of ARD proteins.

The binding and hydroxylation reactions for FIH are:

$$\text{(3.1)}$$

From a derivation analogous to (2.10) – (2.17), we obtain our final expressions for the HIFα CAD-hydroxylation rate (3.4) and the corresponding rate function $v_{FH}$ (3.5) in the presence of competing ankyrin repeats:

$$\frac{dH_{tot}}{dt} = k_p F_{12} = k_p F_{tot} \left( \frac{H}{K_F^p + H_F + P_{tot}} \right) \left( \frac{O_2}{K_M^p + O_2} \right)$$

$$v_{FH} = k_p F_{tot} \left( \frac{1}{K_F^p + H_F + P_{tot}} \right) \left( \frac{O_2}{K_M^p + O_2} \right)$$

$$H_F = \frac{1}{2} \left( \frac{H_{tot} - K_F^p + \sqrt{(K_F^p + F_{tot} - H_F)^2 + 4K_F^p H_F}}{K_F^p} \right)$$

$H$ indicates the total amount of HIFα that is not CAD-hydroxylated. In the absence of competitive inhibition by ARD proteins, HIFα CAD-hydroxylation is given by expressions of identical forms (3.4 – 3.5), but with $K_F^p$ replaced by $K_F^{pA}$. By substituting for $K_F^{pA}$ from (3.2) in the case of $FIH >> H_P$ so that $H_P \approx H$, we see that the HIF-term in (3.4) reduces to the classical form of competitive inhibition:

$$\frac{H}{H + K_F^{pA} + \frac{K_F^{pA}}{K_F} (A + y A_{tot})} = \frac{[S]}{[S] + K_M + \frac{K_M}{K_F} [I]}$$

4. The rate function for Asn-hydroxylation of ankyrin repeats.

Equivalently to (3.3) and using the definition (4.1), $F_a$ can also be expressed as (4.2), and we obtain the ankyrin hydroxylation rate (4.3) and the corresponding rate function $v_{FA}$ (4.4) in the presence of competing HIFα:

$$K_F^{pA} \left( 1 + \frac{H_P}{K_F^p} \right) \equiv K_F^{pA}$$
As for the HIF term in (3.4) where ankyrin repeats were the inhibitors, the ankyrin term in (4.3) reduces to classical competitive inhibition if \( F_{\text{tot}} \gg H_F \), but now HIF\(_\alpha\) is the competitive inhibitor. Finally, to obtain an explicit expression for FIH not bound to ARD proteins, \( F_{\text{free}} \), we use (4.6), which with (3.1), gives the amount of free FIH (4.7).

\[
F_{\text{free}} = F_0 + F_1 + F_2 + F_{12} = (F_0 + F_1) \frac{K_{\text{FIH}}^H + H_F}{K_{\text{FIH}}^H + H_F} \quad 4.6
\]

\[
F_{\text{free}} = F_{\text{tot}} \frac{K_{\text{FIH}}^H + H_F}{K_{\text{FIH}}^H + H_F} \quad 4.7
\]

Figure S4 compares a simulation using the full model with a simulation using Michaelis-Menten kinetics. Because the concentration of the PHDs is assumed low compared to HIF\(_\alpha\), there is not much difference in the levels of total HIF\(_\alpha\) (black curves). The excess of FIH compared to HIF\(_\alpha\) however cause the results to differ more substantially, with the full model giving lower levels of CAD-hydroxylated HIF\(_\alpha\). Moreover, the peak is reached at a higher oxygen concentration (compare red curves). While the differences do not affect any of the conclusions in the present work, the approach we have introduced here will be important for future, more quantitative models of HIF\(_\alpha\) hydroxylation.

5. The Full Model and its non-dimensionalisation.

The full model is given by three differential equations, one each for total HIF\(_\alpha\) (\( H_{\text{tot}} \)), HIF\(_\alpha\) that is not CAD-hydroxylated (\( H \)), and one for unhydroxylated AR (\( A \)). The concentrations of CAD-hydroxylated HIF\(_\alpha\) (\( H_{\text{tot}} \)) and hydroxylated AR (\( A_{\text{tot}} \)) are given by mass conservation of the total amounts, \( H_{\text{tot}} \) and \( A_{\text{tot}} \).

\[
\frac{dH_{\text{tot}}}{dt} = k_d^H - H_{\text{tot}} (k_d^H + v_p) \quad 5.1
\]

\[
\frac{dH}{dt} = k_d^H - H (k_d^H + v_p + v_{pH}) \quad 5.2
\]

\[
\frac{dA}{dt} = k_d^A - A (k_d^A + v_{pA}) \quad 5.3
\]

\[
H_{\text{tot}} = H_{\text{tot}} - H \quad 5.4
\]

\[
A_{\text{tot}} = A_{\text{tot}} - A \quad 5.5
\]

\( k_d \) and \( k_a \) are the basal protein synthesis and degradation rates, respectively, for the species indicated by superscript. We non-dimensionalise the system of ODEs by normalising to the maximally possible amount of HIF\(_\alpha\), and by scaling time with the basal degradation rate constant of HIF\(_\alpha\). Thus, with

\[
H_{\text{tot}}^\text{max} = \frac{k_d^H}{k_d^A} \quad dt = \frac{k_d^H}{k_d^A} \, d\tau \quad \varepsilon = \frac{k_d^H}{k_d^A} \frac{\tau}{\tau_H}
\]

we obtain Eq. 1 – 3 given in the main text. Where “hat” (\( ^\wedge \)) indicates non-dimensional quantities expressed relative to \( H_{\text{tot}}^\text{max} \), and “prime” (\( ^\prime \)) indicates non-dimensional quantities expressed relative to \( k_d^H \). The parameter \( \varepsilon \) is the half life ratio of ARD proteins and HIF\(_\alpha\) under basal turnover conditions, i.e. in the absence of oxygen. We introduce

\[
\dot{O}_2 = \frac{O_2}{K_M} \quad \alpha = \frac{K_{\text{FIH}}}{K_M}
\]

and express the hydroxylation rate functions \( v_p, v_{pH} \) and \( v_{pA} \) as functions of the new non-dimensional variables to obtain the expressions given in the main text (Eq. 4, 6 and 9).


If we assume, as an approximation to experimental data [5], that FIH only does not bind to unhydroxylated but not hydroxylated AR (\( \gamma = 0 \)) and we ignore the presence of HIF\(_\alpha\), we can describe AR-hydroxylation by the differential equation (6.1), which is a simplified version of (5.3). FIH not bound to AR is given by (6.2), which is obtained by employing these assumptions to (4.7).

\[
\frac{dA}{dt} = k_d^A - k_d^A A - k_{\text{rad}}^A F_{\text{tot}} \frac{A}{K_{\text{rad}}^A + A} \frac{O_2}{K_M + O_2} \quad 6.1
\]

\[
F_{\text{free}} = F_{\text{tot}} \frac{K_{\text{rad}}^A}{K_{\text{rad}}^A + A} \quad 6.2
\]

With the definitions

\[
d\tau = k_d^H \, dt \quad \dot{A} = \frac{A}{k_d^A} \quad \beta = \frac{k_{\text{rad}}^A F_{\text{tot}}}{k_d^A A_{\text{tot}}} \quad \kappa = \frac{k_d^H}{K_{\text{rad}}^A k_d^A}
\]

(6.6) and (6.7) can be written in the non-dimensional form given in the main text.

7. References for Additional File 2, Supplementary Methods.


