

**Supplemental Material for**  
**The second coordination sphere of FIH controls hydroxylation**

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### 1. Thermal stability of FIH variants

The thermal stability of FIH variants was measured by differential scanning calorimetry, as described in the text.

**Table S1.** DSC melting temperature analysis of FIH and its mutants.

	$T_{M(\text{app})}$ (°C)
WT FIH	54.5
N205A	58.6
N294A	58.9
R238M	56.7
Q239N	56.2

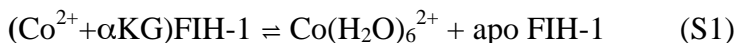
FIH (50  $\mu\text{M}$ ) in 50 mM HEPES pH 7.50, temperature range: 25-75 °C, scan rate: 60 °C/hour.

### 2. Co<sup>II</sup> binding thermodynamics to FIH variants.

#### Metal titration data fitting:

The binding affinity of (Co<sup>2+</sup>+ $\alpha$ KG)FIH for Co<sup>2+</sup> was obtained by competitive titration in 50 mM HEPES, pH 7.50 at 23°C. A 200  $\mu\text{L}$  solution of apo FIH (20  $\mu\text{M}$ ), citrate (1.00 mM), and  $\alpha$ KG (100  $\mu\text{M}$ ) was temperature equilibrated in a fluorescence cuvette, while a separate solution of CoCl<sub>2</sub> (1.00 mM) with citrate (1.00 mM) in buffer was loaded into a titrating syringe. As FIH-1 utilizes  $\alpha$ KG as a co-substrate as well as a bidentate ligand for metal,  $\alpha$ KG was included to complete the relevant ligand set of (Co<sup>2+</sup>+ $\alpha$ KG)FIH. This experiment used citrate to buffer the concentration of free Co<sup>2+</sup>, or Co(H<sub>2</sub>O)<sub>6</sub><sup>2+</sup>, as the log  $\beta$  values for Co(II) binding to citrate are well defined.<sup>1</sup> Upon addition of small volumes of CoCl<sub>2</sub>, apo FIH bound the available  $\alpha$ KG and Co<sup>2+</sup>.

The fluorescence intensity of FIH-1 at 340 nm ( $F_{340}$ ) was plotted against  $\log[\text{Co}^{2+}]_{\text{free}}$ , exhibiting the sigmoidal shape characteristic of a binding equilibrium. The binding curve was fitted to a simple 1:1 binding equilibrium in which the  $\text{Co}^{2+}$  dissociation constant of  $(\text{Co}^{2+} + \alpha\text{KG})\text{FIH}$  ( $K_D$ ) is with respect to  $\text{Co}(\text{H}_2\text{O})_6^{2+}$  (Eq. 1). The fluorescence intensity ( $F_{340}$ ) was fitted to Eq. S2<sup>2</sup> which yielded  $\log K_D$  ( $x$ ) relative to the  $\log[\text{Co}^{2+}]_{\text{free}}$  ( $c$ ), using a cooperativity parameter ( $B$ ) which accounted for both chemical cooperativity involved with dimeric FIH-1 as well as the spectroscopic cooperativity involved with fluorescence signal from the eight Trp residues of FIH. The results are in Table S2.



$$F_{340} = F_{\min} + (F_{\max} - F_{\min}) / (1 + 10^{(c-x)B}) \quad (\text{S2})$$

**Table S2.**  $\text{Co}^{\text{II}}$  binding affinity of FIH-1 mutants in the presence of  $\alpha\text{KG}$

	$K_D$ (M)	$B$
WT FIH	$1.38(6) \times 10^{-7}$	$2.2 \pm 0.2$
N205A	$1.63(3) \times 10^{-7}$	$1.6 \pm 0.1$
N294A	$1.01(4) \times 10^{-7}$	$2.3 \pm 0.2$
Q239N	$1.45(8) \times 10^{-7}$	$1.9 \pm 0.2$
R238M	$1.9(2) \times 10^{-7}$	$1.3 \pm 0.1$

$\text{CoCl}_2$  (1 mM) / citrate (1 mM) was titrated into FIH (20  $\mu\text{M}$ ),  $\alpha\text{KG}$  (100  $\mu\text{M}$ ) in 50 mM HEPES pH 7.50.

## References

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- <sup>1</sup> Martell, A. E., and Smith, R. M. (eds). (1974) *Critical stability constants*, Plenum Press, New York  
<sup>2</sup> Mills, S. A., and Marletta, M. A. (2005) *Biochemistry* **44**, 13553-13559