

Supplemental Material for

Substrate Positioning by Gln²³⁹ Stimulates Turnover in FIH, an α KG-Dependent Hydroxylase

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1. Thermal Stability of FIH and Gln²³⁹→X Variants
2. Intrinsic Tryptophan Fluorescence

1. Differential Scanning Calorimetry

The thermal stability of all the Gln²³⁹→X variants was determined using a MicroCal VP-DSC Microcalorimeter. FIH (30 μM) was reconstituted with CoCl₂ (30 μM) and αKG (400 μM) in 50 mM HEPES pH 7.00. Samples were heated in the calorimeter over a 10-70°C range with a scan rate of 30 °C/h. Thermal unfolding of FIH was irreversible, and the apparent melting temperature ($T_{M(\text{app})}$) was taken as the peak in the heat capacity data. To correct for baseline drift, a buffer scan was subtracted from each data set.

Table S1 Melting temperatures determined using DSC for Gln²³⁹→X variants

	$T_{M(\text{app})}$ (°C)
WT FIH ^a	54.5
Q239A	54.3
Q239N ^a	56.2
Q239H	53.2
Q239E	51.9
Q239L	53.3

FIH (30μM) in 50 mM HEPES pH 7.00

- a. Saban, E., Chen, Y.-H., Hangasky, J., Taabazuing, C., Holmes, B., and Knapp, M. (2011) The second coordination sphere of FIH controls hydroxylation, *Biochemistry* 50, 4733-4740.

2. Intrinsic Tryptophan Fluorescence

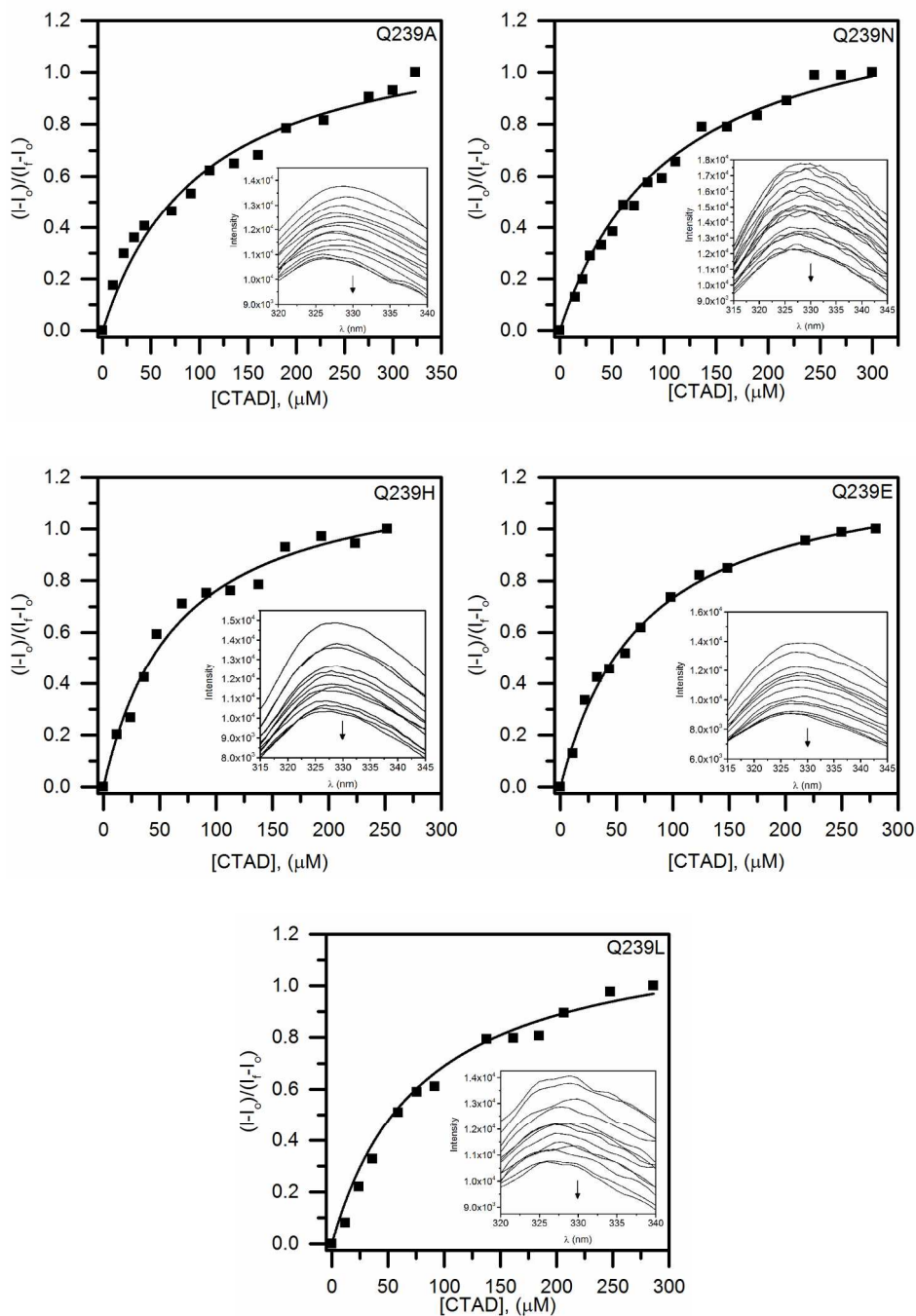


Figure S1 Intrinsic tryptophan fluorescence. Normalized fluorescence intensity at 330 nm versus total [CTAD]. FIH (1.5 μM), CoCl_2 (25 μM) and αKG (500 μM) in 50 mM HEPES pH 7.05 was titrated with a CTAD stock (1.00 mM) containing FIH (1.5 μM), CoCl_2 (25 μM) and αKG (500 μM) in 50 mM HEPES pH 7.05. Inset: Fluorescence emission spectra collected after each addition of CTAD.