

Supplementary Materials
for
Redox Control of Iodotyrosine Deiodinase

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Table S1. Data collection and refinement statistics for T239A HslYD-F-Tyr.

Protein	T239A HslYD-F-Tyr
Protein Data Bank code	5YAK
Ligands	FMN and F-Tyr
Data collection	
Space group	$P6_1$
No. Molecule/ASU	6
Unit cell parameters (\AA , $^\circ$)	$a=105.08, b=105.08, c=300.20$ $\alpha=90, \beta=90, \gamma=120$
Resolution range (\AA) ^a	46.5-2.30 (2.36-2.30)
No. of observed reflections	661766
No. of unique reflections ^a	82734 (5790)
Completeness ^a	100 (99.8)
Redundancy ^a	8.0 (8.2)
R_{sym} (%) ^a	9.3 (35.1)
$I/\sigma I$ ^a	41.5 (6.5)
Wilson B factor (\AA^2)	37.5
Refinement	
Resolution used in refinement (\AA)	46.52-2.30
No. of reflections used in working set	78650
No. of reflections for R_{free} calculation	4144
R_{work} (%), R_{free} (%)	18.3, 22.6
No. atoms	
Protein	11102 (A-F)
Heteroatoms	186/84 (FMN and F-Tyr)
Solvent	386
Mean B -factor (\AA^2)	
Protein	48.8
FMN	38.3
F-Tyr	37.7
solvent	42.7
RMSD from ideality	
Bond lengths (\AA)	0.012
Bond angles ($^\circ$)	1.5
Ramachandran analysis	
Favored (%)	97.5
Allowed (%)	2.5
Outliers	0

^aThe values in parentheses are for the highest resolution shell.

^bThe F-Tyr in PDB is YOF.

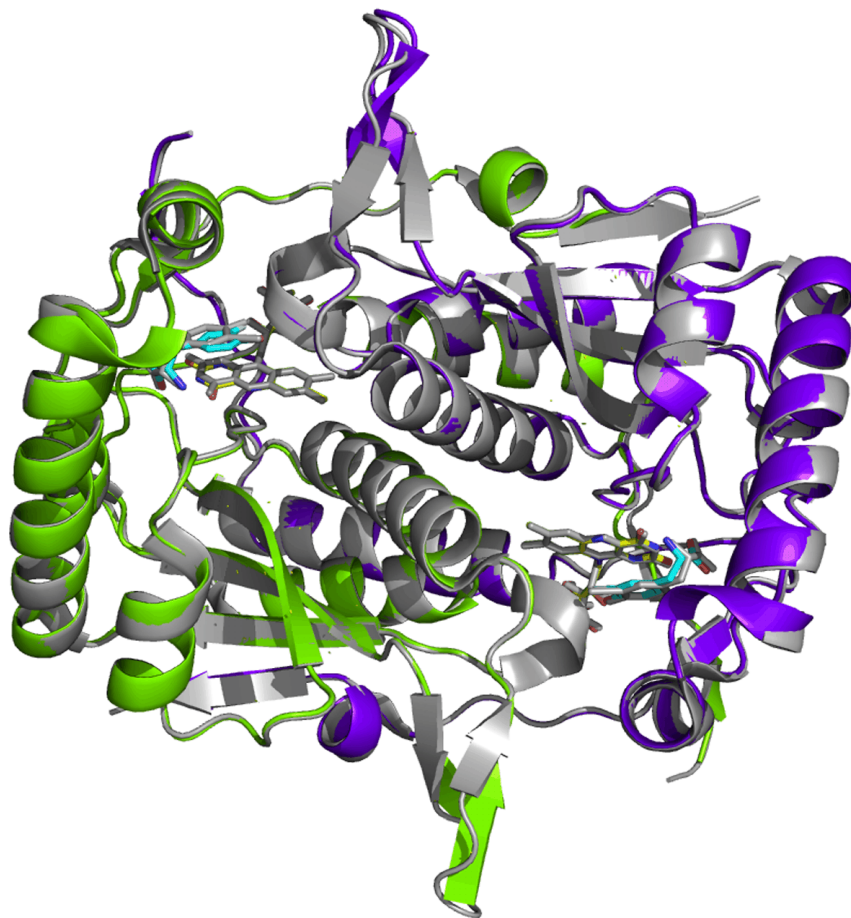


Figure S1. Structural comparisons between two forms of HsIYD with active site ligands. The co-crystal structure of HsIYD T239A (green and purple polypeptides of the α_2 -homodimer) and F-Tyr (carbons in cyan) (pdb 5YAK) is overlaid with the co-crystal structure of wild type HsIYD excluding the N-terminal transmembrane sequence, grey) and I-Tyr (grey) (pdb 4TTC).¹

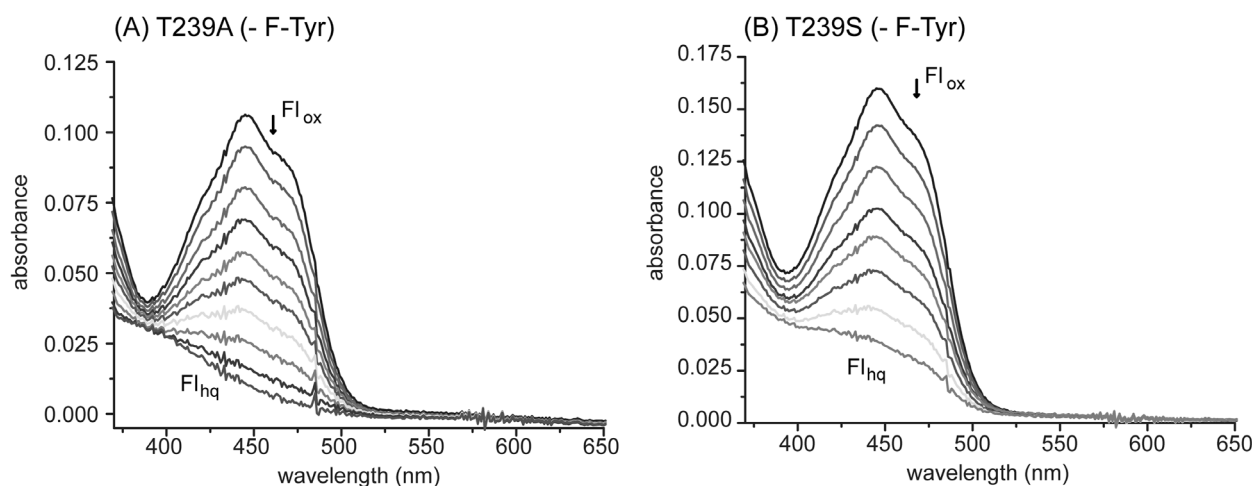


Figure S2. Reduction of (A) T239A and (B) T239S variants of HsIYD in the absence of F-Tyr. Solutions containing 900 μM xanthine, 2 μM methyl viologen, 200 mM potassium chloride and 100 mM potassium phosphate pH 7.4 were degassed with argon and reduction was initiated by addition of xanthine oxidase (40 $\mu\text{g}/\text{ml}$).^{2,3}

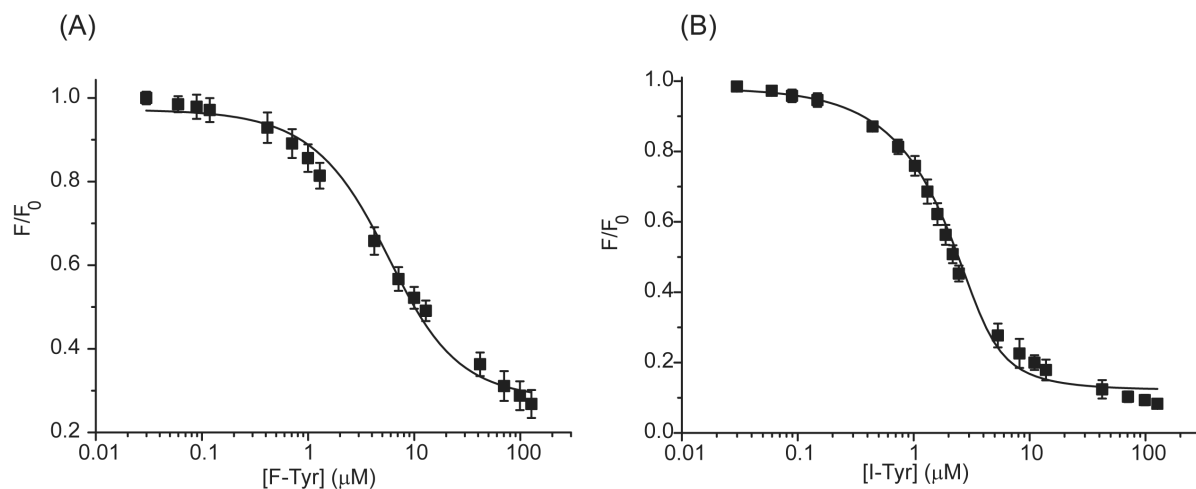


Figure S3. Affinity of the T239A variant of HsIYD for I-Tyr and F-Tyr. IYD (T239A, 4.5 μM) in 100 mM potassium phosphate pH 7.4 (25 $^{\circ}\text{C}$) was alternatively titrated with the indicated concentrations of (A) F-Tyr and (B) I-Tyr. Fluorescence ($\lambda_{\text{ex}} = 450 \text{ nm}$, $\lambda_{\text{em}} = 527 \text{ nm}$) of the active site Fl_{ox} was recorded and the ratio of emission observed (F) over the initial fluorescence (F_0) was used to calculate K_d values as published previously.^{4,5} Data points represent the average of three independent measurements and error bars indicate the standard deviation. The line indicates the non-linear best fit of the data.

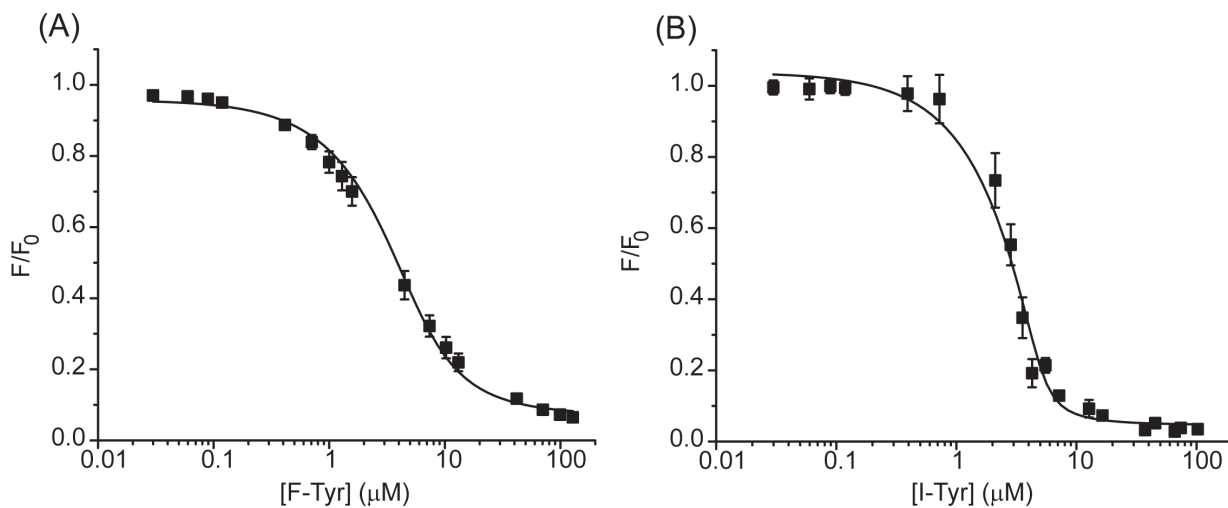


Figure S4. Affinity of the T239S variant of HsIYD for F-Tyr and I-Tyr. IYD (T239S, 4.5 μM) in 100 mM potassium phosphate pH 7.4 (25 $^{\circ}\text{C}$) was alternatively titrated with the indicated concentrations of (A) F-Tyr and (B) I-Tyr. Fluorescence ($\lambda_{\text{ex}} = 450 \text{ nm}$, $\lambda_{\text{em}} = 527 \text{ nm}$) of the active site Fl_{ox} was recorded and the ratio of emission observed (F) over the initial fluorescence (F_0) was used to calculate K_d values as published previously.^{4,5} Data points represent the average of three independent measurements and error bars indicate the standard deviation. The line indicates the non-linear best fit of the data.

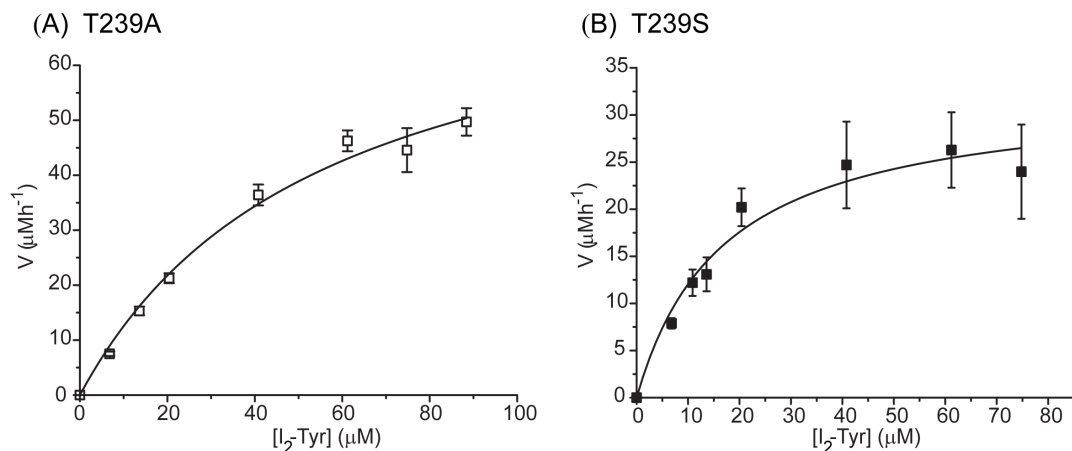
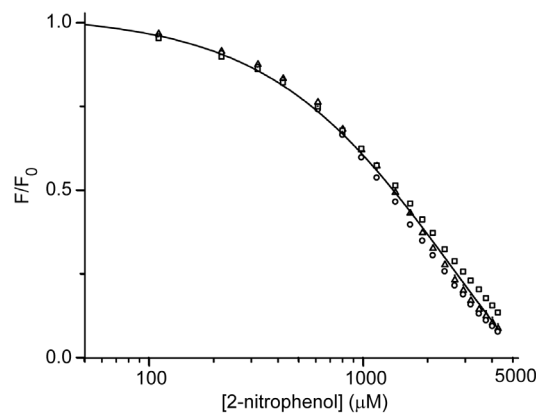


Figure S5. Catalytic deiodination of I₂-Tyr by HsIYD T239A and T239S. Rates of deiodination were measured by release of [¹²⁵I]-iodide from [¹²⁵I]-I₂-Tyr as described previously.^{6,7} HsIYD variants (A) T239A (0.4 μM) and (B) T239S (0.08 μM) were incubated at 20 °C with the indicated concentrations of [¹²⁵I]-I₂-Tyr in 0.50 mM methimidazole, 50 mM 2-mercaptoethanol, 200 mM KCl, 100 mM potassium phosphate pH 7.4. Reaction was initiated by addition of 1% sodium dithionite (from a 10% stock in 5% sodium bicarbonate) and quenched after 10 min by addition of 100 μl of 0.1 % I₂-Tyr in 0.1 M NaOH. [¹²⁵I]-iodide was separated by ion exchange (AG50W-X8 resin) and quantified by scintillation counting. The average of the three independent measurements was fit to the Michaelis-Menten equation using Origin 7.0 (line) and the error represents the standard derivation of the three measurements.

Figure S6. Affinity of 2-nitrophenol for WT HsIYD. HsIYD (3 μM) in 100 mM MES pH 6.0, 500 mM NaCl and 10% glycerol was titrated with the indicated concentrations of 2-nitrophenol. Fluorescence ($\lambda_{\text{ex}} = 450 \text{ nm}$, $\lambda_{\text{em}} = 516 \text{ nm}$) of the active site Fl_{ox} was recorded and the ratio of emission observed (F) over the initial fluorescence (F₀) was used to calculate the K_d value for 2-nitrophenol as published previously.^{4,5} The line indicates the non-linear best fit of three independent sets of data.



References

1. Hu J, Chuenchor W, Rokita SE (2015) A switch between one- and two-electron chemistry of the human flavoprotein iodotyrosine deiodinase is controlled by substrate. *J. Biol. Chem.* 290:590-600.
2. Massey V. 1991. A simple method for the determination of redox potentials. In *Flavins Flavoproteins Proc. Int. Symp.* (eds. B. Curti, S. Ronchi, and G. Zanetti), pp. 59-66. Gruyter & Co., Berlin.
3. van den Heuvel RHH, Fraaije MW, Van Berkel WJH (2002) Redox properties of vanillyl-alcohol oxidase. *Methods Enzymol.* 353:177-186.
4. Warner JR, Copley SD (2007) Pre-steady-state kinetic studies of the reductive dehalogenation catalyzed by tetrachlorohydroquinone dehalogenase. *Biochemistry* 46:13211-13222.
5. McTamney PM, Rokita SE (2009) A mammalian reductive deiodinase has broad power to dehalogenate chlorinated and brominated substrates. *J. Am. Chem. Soc.* 131:14212-14213.
6. Rosenberg IN, Goswami A (1984) Iodotyrosine deiodinase from bovine thyroid. *Methods Enzymol.* 107:488-500.
7. Friedman JE, Watson JA, Jr., Lam DW-H, Rokita SE (2006) Iodotyrosine deiodinase is the first mammalian member of the NADH oxidase / flavin reductase superfamily. *J. Biol. Chem.* 281:2812-2819.