Supplemental Information for

Iodotyrosine Deiodinase: A Unique Flavoprotein Present in Organisms of Diverse Phyla

Abhishek Phatarphekar,[1] Jennifer M. Buss[2] and Steven E. Rokita[1]

[1]From the Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218
[2]Department of Chemistry and Biochemistry, University of Maryland,
College Park, MD 20742 USA

Table S1. NCBI accession numbers for IYD homologs chosen for expression.

IYD homolog	Protein accession	Derived from Nucleotide/genome accession	Amino acids deleted[a]
Zebrafish (drIYD)	XP_696511.1	XM_691419.3	2-39
Lancelet (bflYD)	XP_002610029.1	XM_002609983.1	2-34
Honeybee (amIYD)	XP_397179.2	XM_397179.4	2-41
Daphnia (dpIYD)	EFX90111.1	GL732523.1[b,c]	none
Sea anemone (nvIYD)	XP_001633169.1	XM_001633119.1	none
Hydra (hmIYD)[d]	XP_002164528.1	XM_002164492.1	none
Bacteria (hhIYD)	YP_004447048.1	CP002691.1[e]	none

[a]Amino acids predicted to act as the N-terminal membrane domain were removed to express soluble protein.
[b]Gene sequence is obtained by joining bases 2920261 to 2920660, 2920724 to 2920849, 2920912 to 2921102 and 2921161 to 2921346 of the *D. pulex* genome.
[c]Base at position 2920782 (base 459 of gene sequence) was changed from C to T making a silent mutation to remove XhoI site in native sequence.
[d]The protein and nucleotide sequences were recently superseded by XP_002164528.2 and XM_002164492.2 respectively after this investigation was completed. The updated protein sequence showed variation in the first 42 amino acids compared to the expressed protein sequence.
[e]Gene sequence is obtained by joining bases 2972330 to 2972998 of the *H. hydrossis* genome.

Table S2. Optimized conditions for expressing IYD homologs.

IYD homolog	IPTG added (μM)	Induction temp (ºC)	Induction time (hrs)
Zebrafish (drIYD)	400	18	4
Lancelet (bflYD)	50	18	12
Honeybee (amIYD)	400	18	4
Daphnia (dpIYD)	50	16	12
Sea anemone (nvIYD)	400	18	4
Hydra (hmIYD)	20	16	12
Bacteria (hhIYD)	400	18	4

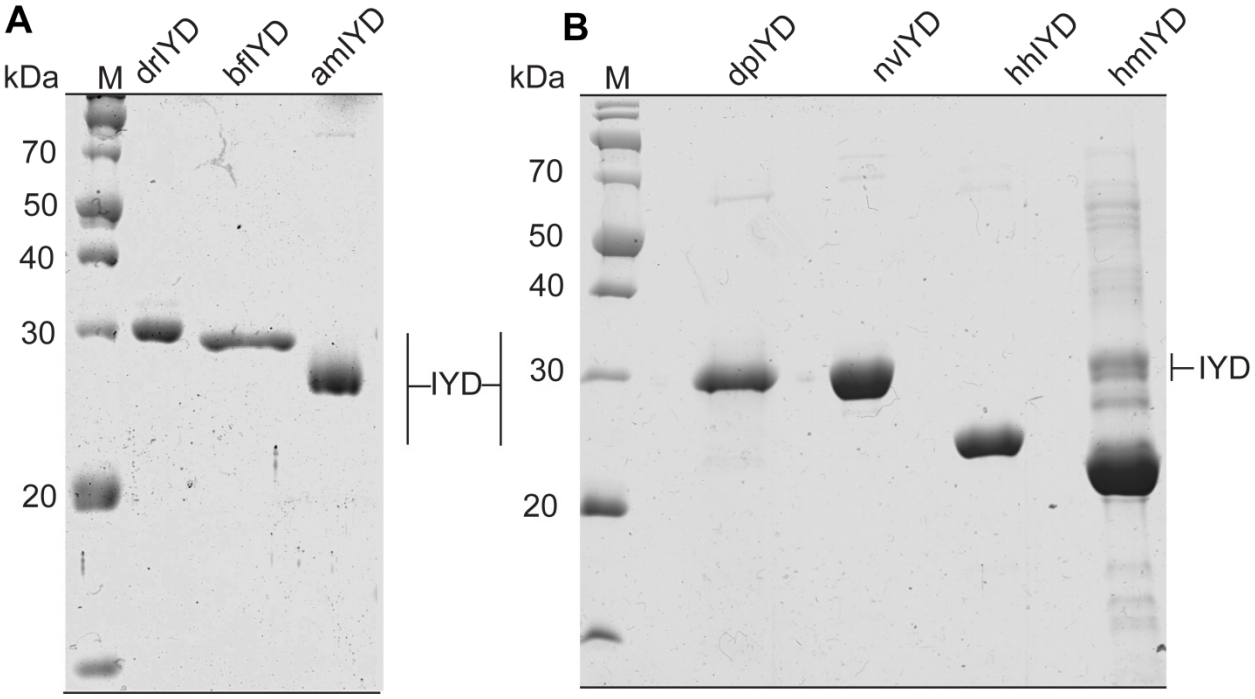

Figure S1. SDS-PAGE gel images of purified IYD homologs. A. IYD homologs from zebrafish (drIYD), lancelet (bfIYD) and honeybee (amIYD). B. IYD homologs from daphnia (dpIYD), sea anemone (nvIYD), hydra (hmIYD) and the bacteria *H. hydrossis* (hhIYD). M is the marker lane with molecular weights of protein markers indicated on the left in kDa.

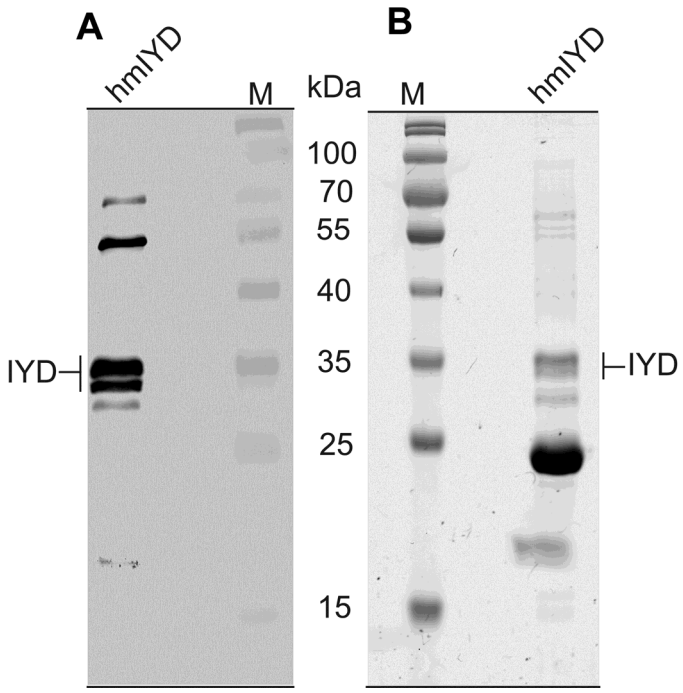

Figure S2. Identification of active IYD from hydra (hmIYD). A. Western blot of the active protein fraction from hmIYD purification was analysed by using a His-tag mouse monoclonal antibody and goat anti mouse antibody performed as per manufacturer procedures (Novagen). The 55 kDa band is the SUMO-hmIYD fusion which exhibited no deiodinase activity (data not shown). B. Coomassie stained SDS-PAGE image of same active protein fraction tested in panel A.

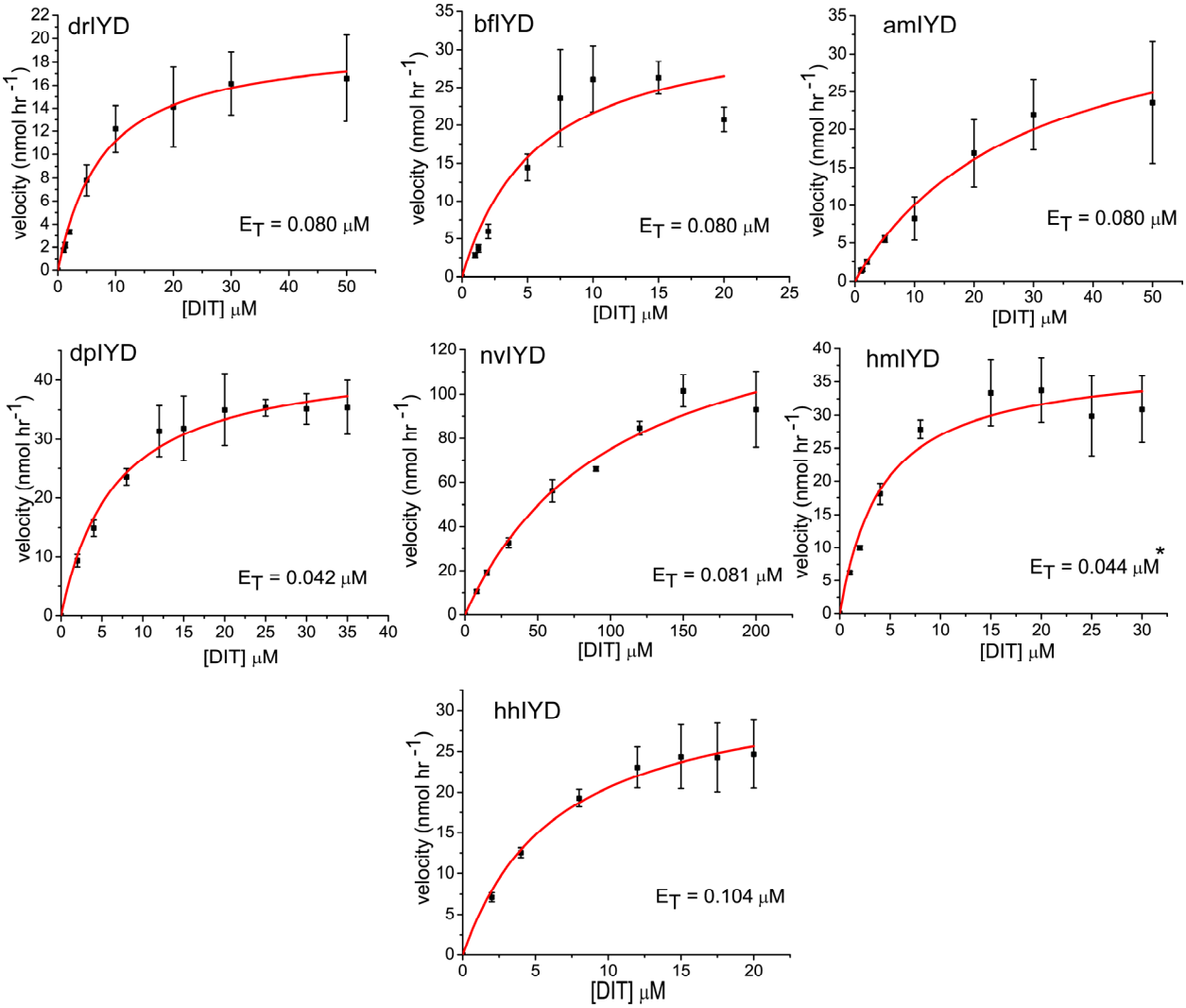

Figure S3. Deiodination rate dependence on DIT concentration for IYD homologs. Kinetic constants were determined by fitting initial rates to the Michealis-Menten equation (red curve) using Origin 7.0. Each data point represents an average of 3 individual observations, and the error bars represent their standard deviation. E_T indicates the total enzyme concentration used for each IYD homolog. Estimated enzyme concentration is indicated by (*) (see experimental procedures).