## Substrate-Protein Interaction in Human Tryptophan

## Dioxygenase: The Critical Role of H76

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**Supporting Information Available**: Complete Ref. 21 and supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org."

Complete Ref.21: Forouhar, F.; Anderson, J. L.; Mowat, C. G.; Vorobiev, S. M.; Hussain, A.; Abashidze, M.; Bruckmann, C.; Thackray, S. J.; Seetharaman, J.; Tucker, T.; Xiao, R.; Ma, L. C.; Zhao, L.; Acton, T. B.; Montelione, G. T.; Chapman, S. K.; Tong, L., Molecular insights into substrate recognition and catalysis by tryptophan 2,3-dioxygenase. Proc Natl Acad Sci U S A 2007, 104, (2), 473-8.

	1 10	20	30	40	50	60	70	80	90	100	110	120	130
HumanTDO MouseTDO RattusTDO XanthomonasTDO PseudomonasTDO	MSGCPFLGNNFGYT MSGCPFAGNSVGYT MSGCPFSGNSVGYT	ifkklpveg ilknysmed ilknlsmed mpvdki msqcpfssi	SEEDKSQTGYNF IEEDRAQTGYNF IEEDGAQTGYNF ILRD-LEPGIH1 IPPA-EHHNAEL	RASKGGLIYE RASKGGLIYE RASKGGLIYE IDLEGRLIYE INFSDSHSYE	NYLHLEKVLN Nylqlekiln Dylqlekiln Gylrldqlls Dyldlgrils	NAQELQSETKO NAQELQSEVKO NAQELQSEIKO SAQQPLSE SAQHPLSP	NKIHDEHLF NKIHDEHLF NKIHDEHLF PAHHDEHLF DHNEHLF	FIITHQAYELA FIITHQAYELA FIITHQAYELA FIIQHQTSELA FIIQHQTSELA	IFKQILHELD IFKQILHELD IFKQILHELD ILKLLAHELR IMKLMLHELK	SVREIFQNGH SVREIFQNGH SVREIFQNGH SVREIFQNGH AAIVHLQRDE AAREHVRQGQL	/RDERNMLKV /RDERNMLKV /RDERNMLKV /HQCRKVLA- _PPAFKMLA-	VSRMHRVSVILK IARMHRVVVIFK MTRMHRVVVIFK RSKQVLR RVSRIFD	LLVQQFS LLVQQFS LLVQQFS QLTEQHS QLVHANA
	131 140	150	160	170	180	190	200	210	220	230	240	250	260
HunanTDO MouseTDO RattusTDO XanthononasTDO PseudononasTDO	I ILETHTALDFNDFF VLETHTALDFNDFF VLETHTALDFNDFF VLETLTPSEYHGFF VLATHTPSEYKSIF	REYLSPASG REYLSPASG REYLSPASG REYLSPASG RDYLGPSSG RPYLGQSSG	QSLQFRLLEN QSLQFRLLEN QSLQFRLLEN QSLQYRYIEFL QSLQYRYIEFI QSFQYREIEFJ	(IGVLQNHRV (IGVLQSLRV (IGVLQSLRV (IGVLQSLRV (IG	PYNRRHYRDN Pynrkhyrdn Pynrkhyrdn	NFKGEENELLL NFGGDYNELLL NFEGDYNELLL NKNPQMLQ NKSAALLF	KSEQEKTLI KSEQEQTLI KSEQEQTLI VFAYDPAGO PHAHRPELI	LELVEANLERT LQLVEANLERT LQLVEANLERT QARLREYLE LQSLEASTA	PGLEPHGFN PGLEPNGFN PGLEPHGFN	FNGKLEKNITF FNGKFEKNILK FNGKFEKNILK RF TF	RGLEEEFIRI KGLEEEFLRI KGLEEEFLRI KGLEEEFLRI KGLEEEFLRI SHYDEAIAL	QAKEESEEKEEQ Qaktdseekeeq Qakkdseekeeq Larfghaipqqy Marsglsidpar	HAEFQKQ HAEFRKQ HAEFRKQ QARDHTA LALDSTT
	261 270	280	290	300	310	320	330	340	350	360	370	380	390
HunanTDO HouseTDO RattusTDO XanthononasTDO PseudononasTDO	261 270 I KEVLLSLFDEKRHG KEVLLCLFDEKRHG KEVLLCLFDEKRHG AHVAU TTQHG	280 HLLSKGER YLLSKGER YLLSKGER DTLRPYFE PSYEARHR	290 RLSYRALQGALT RLSYRALQGALT RLSYRALQGALT RLSYRALQGALT RL	300 IIYFYREEPF IIYFYREEPF IIYFYREEPF YENTDF YENTDF	310 FQVPFQLLTS FQVPFQLLTS FQVPFQLLTS YHREYSLCED YHRLYQLAEK	320 SLMDIDSLMTK SLMDIDTLMTK SLMDIDTLMTK SLMDIDTLMTK DLVDVETQFQL KFIDLEDSFRQ	330 CHRYNHYCH CHRYNHYCH CHRYNHYCH CHRYNHYCH CHRFRHHRT CHRFRHYTT	340 VHRHLGSKAGT VHRHLGTKAGT VHRHLGSKAGT VHRVLGFKRGT VERLIGFQPGT	350 GGSSGYHYL GGSSGYHYL GGSSGYGFL GGTEGVGYL	360 RSTYSDRYKYF RSTYSDRYKYF RSTYSDRYKYF QQALALTFF RKHLDTYLF	370 VOLFNLSTY VOLFNLSTY VOLFNLSSY PELFDVRTS PELHRVRSS	380 LIPRHHIPKHNP LYPRHHVPKHNP LYPRHHIPKHNP VGYDNRPPQGSA L	390 TIHKFLY TIHKFLY TIHKFLY DAGKR

Figure S1. Sequence alignment of hTDO with TDOs from other organisms. The DNA sequences from human (*H. sapiens*), mouse (*M. musculus*), rat (*R. norvegicus*), and bacteria (*X. campestris* and *P. fluorescens*) are aligned with a web-based software (Multalin version 5.4.1.).<sup>55</sup> The residues labeled in red and blue indicate 90% and 50% consensus levels, respectively. The truncation positions for the hTDO used for this work are indicated by the arrows.



Figure S2. Characterization of the purified hTDO protein sample. In (A), the 12 % SDS-PAGE data show the purified hTDO loaded in increasing concentrations (lanes 1-7), demonstrating the purity of the enzyme. The lane labeled as M is from molecular markers (Bio-Rad); the 37, 50, 75 and 100 kD bands are used as molecular weight references. For the hIDO sample, a higher molecular weight band visible at ~ 85 kD is attributed to residual dimeric form of hTDO. Similar bands have been reported for rat liver TDO.<sup>35, 56</sup> The assignment of the bands to hTDO were confirmed by western blot analysis, with an anti-histidine antibody (Roche). In (B), the elution profile (monitored at 407 nm) of the purified protein obtained from a size-exclusion column (Superdex 200) shows that the majority of the enzyme elutes at ~12 ml, confirming that the enzyme was purified to near homogeneity.



Figure S3. (A) The <sup>12</sup>C<sup>16</sup>O-<sup>13</sup>C<sup>18</sup>O isotope difference spectra associated with the  $v_{Fe-CO}$  modes of the wild type and the H76S and H76A mutants of hTDO in the presence and absence of Trp or Trp analogs. (B) The  $v_{C-O}$  modes of the wild type and the H76S and H76A mutants of L-Trpbound hTDO, and their associated <sup>12</sup>C<sup>16</sup>O-<sup>13</sup>C<sup>18</sup>O isotope difference spectra. The conditions are the same as those described in Fig. 7. In the difference spectra all the heme modes are cancelled out; the remaining positive and negative peaks are associated with <sup>12</sup>C<sup>16</sup>O and <sup>13</sup>C<sup>18</sup>O, respectively. Isotope difference spectra of the substrate-free and L-Trp-bound wild type hTDO are not shown, as they have been reported elsewhere.<sup>24</sup> The numbers labeled in gray in (A) are the associated Fe-C-O bending modes ( $\delta_{Fe-C-O}$ ).



**Figure S4. The postulated dioxygenase mechanisms of TDO and IDO.** The mechanisms are described in the text.