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.

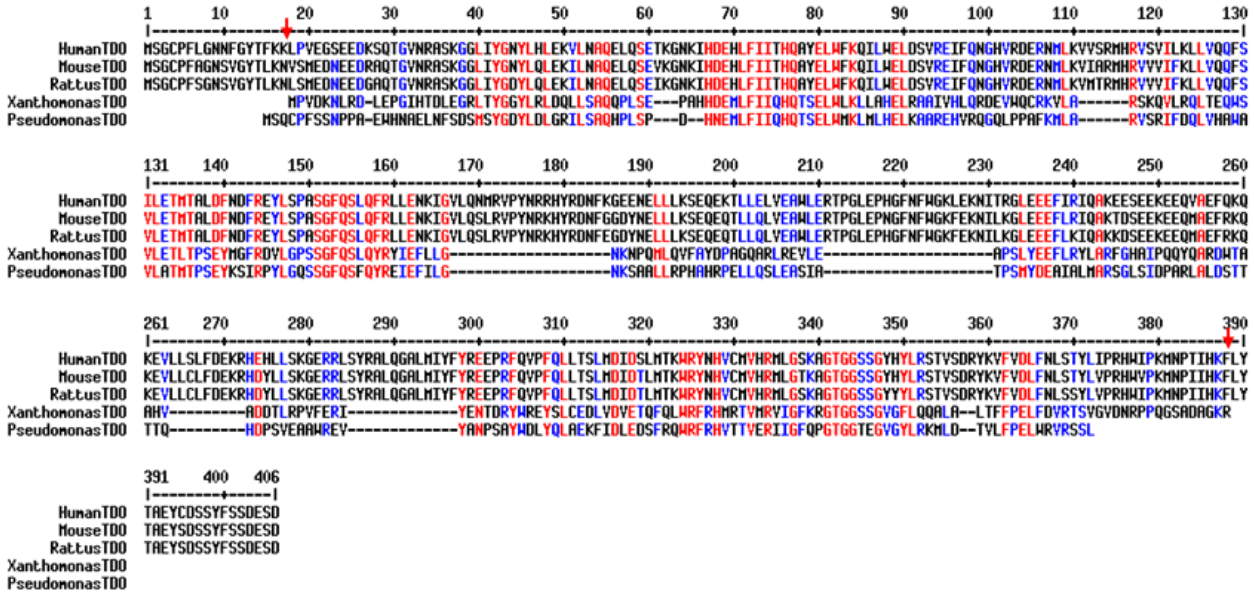

Figure S1. Sequence alignment of hTDO with TDOs from other organisms. The DNA sequences from human (*H. sapiens*), mouse (*M. musculus*), rat (*R. norvegicus*), and bacteria (*X. campestris* and *P. fluorescens*) are aligned with a web-based software (Multalin version 5.4.1.).[55] The residues labeled 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.

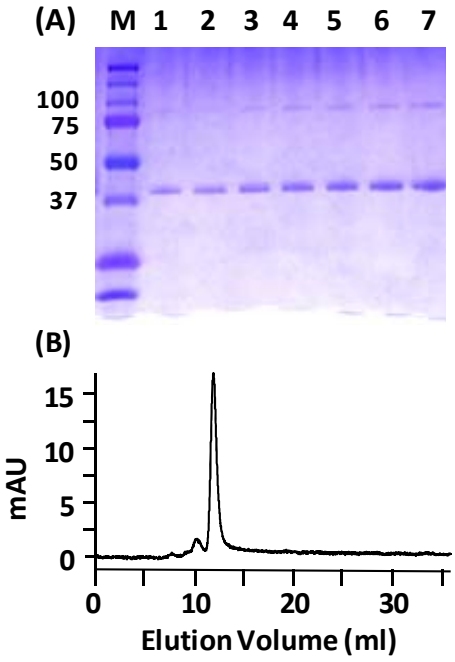

Figure S2. Characterization of the purified hTDO protein sample. In (A), the 12 % SDS-PAGE data show the purified hTDO loaded in increasing concentrations (lanes 1-7), demonstrating the purity of the enzyme. The lane labeled as M is from molecular markers (Bio-Rad); the 37, 50, 75 and 100 kD bands are 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.[35, 56] 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.

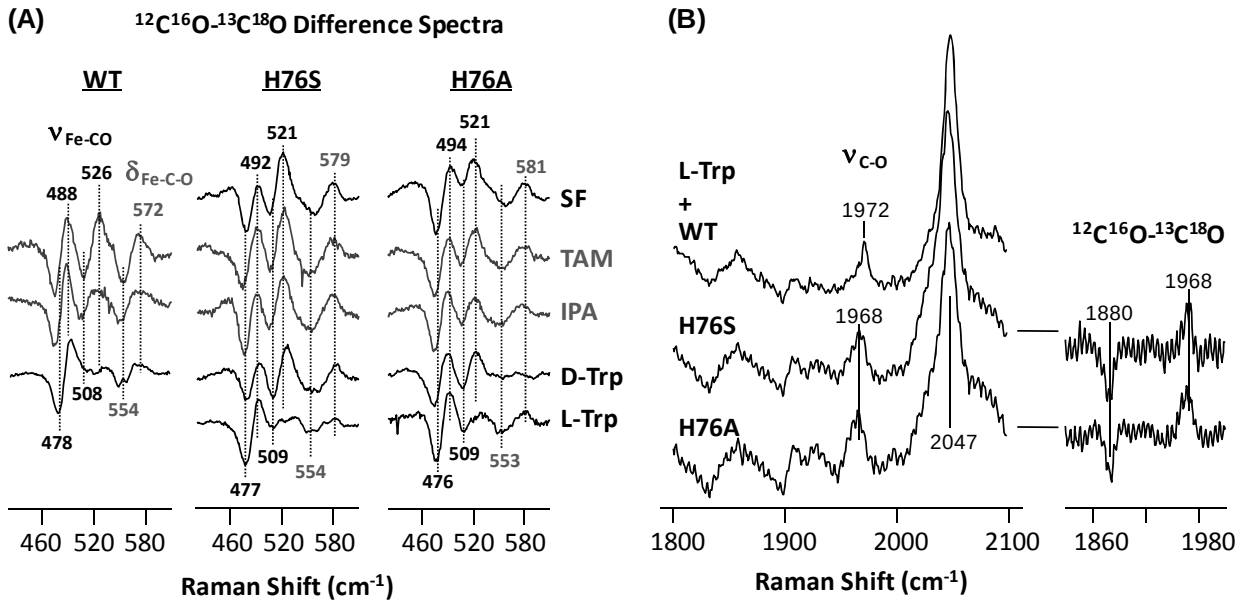

Figure S3. (A) The $^{12}C^{16}O$-$^{13}C^{18}O$ isotope difference spectra associated with the ν_{Fe-CO} modes of the wild type and the H76S and H76A mutants of hTDO in the presence and absence of Trp or Trp analogs. (B) The ν_{C-O} modes of the wild type and the H76S and H76A mutants of L-Trp-bound hTDO, and their associated $^{12}C^{16}O$-$^{13}C^{18}O$ 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 $^{12}C^{16}O$ and $^{13}C^{18}O$, respectively. Isotope difference spectra of the substrate-free and L-Trp-bound wild type hTDO are not shown, as they have been reported elsewhere.[24] The numbers labeled in gray in (A) are the associated Fe-C-O bending modes (δ_{Fe-C-O}).

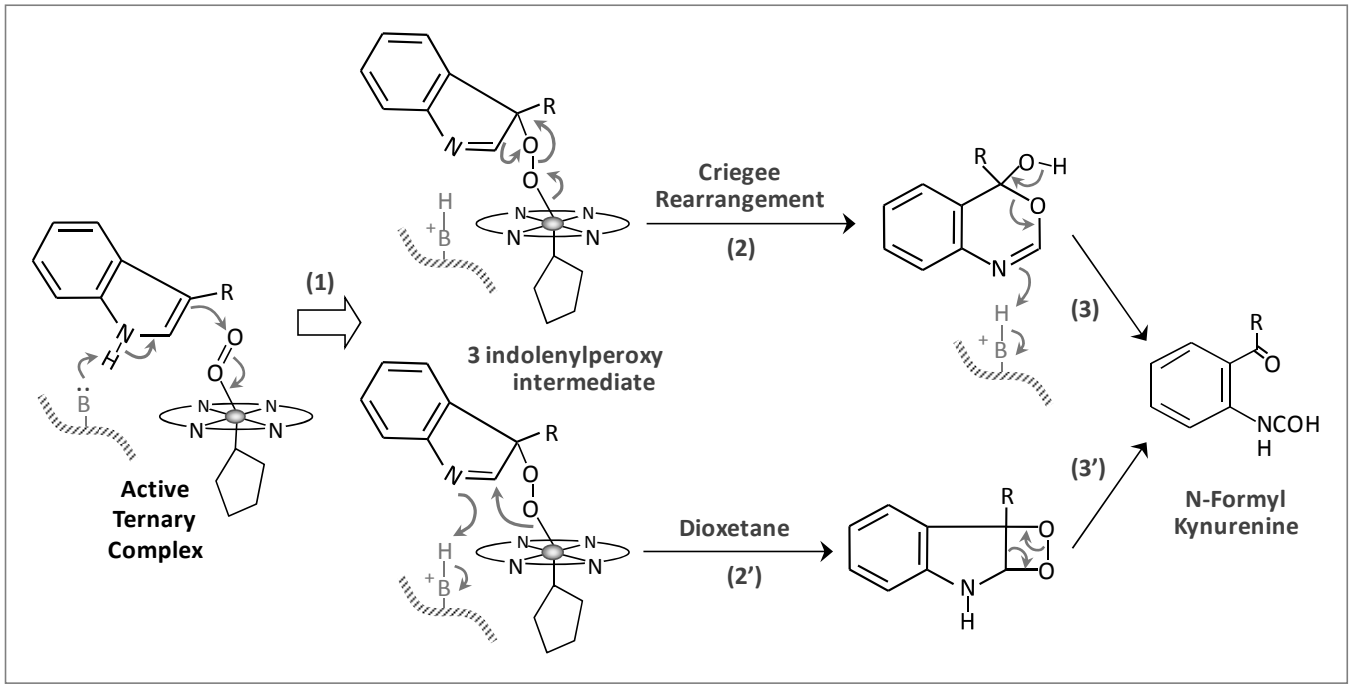

Figure S4. The postulated dioxygenase mechanisms of TDO and IDO. The mechanisms are described in the text.