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Supporting information for article:

Structures of L-proline *trans*-hydroxylase reveal the catalytic specificity and provide deeper insight into AKG-dependent hydroxylation

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Table S1 Ligand concentrations for soaking crystals.

	Fe(NH ₄) ₂ (SO ₄) ₂	AKG	L-proline	3-Hyp	4-Hyp
UbPH-apo	/	/	/	/	/
UbPH-AKG	10 mM	10 mM	/	/	/
UbPH-LP	10 mM	10 mM	10 mM	/	/
UbPH-4Hyp	10 mM	10 mM	/	/	10 mM
UbPH-3Hyp	10 mM	10 mM	/	10 mM	/

Table S2 The results of the DALI search.

Chain	Z	rmsd	lali	nres	%id	PDB	Description
5nci-A	35.8	1.5	251	263	41	MOLECULE:	Leucine Hydroxylase
5l9b-A	14.6	3.2	185	222	15	MOLECULE:	Egl Nine Homolog 1
5c5t-A	12.3	3	153	181	12	MOLECULE:	Prolyl 4-hydroxylase

The UbPH-apo structure was used as a model to search in the database.

Only the three structures used in this article are shown.

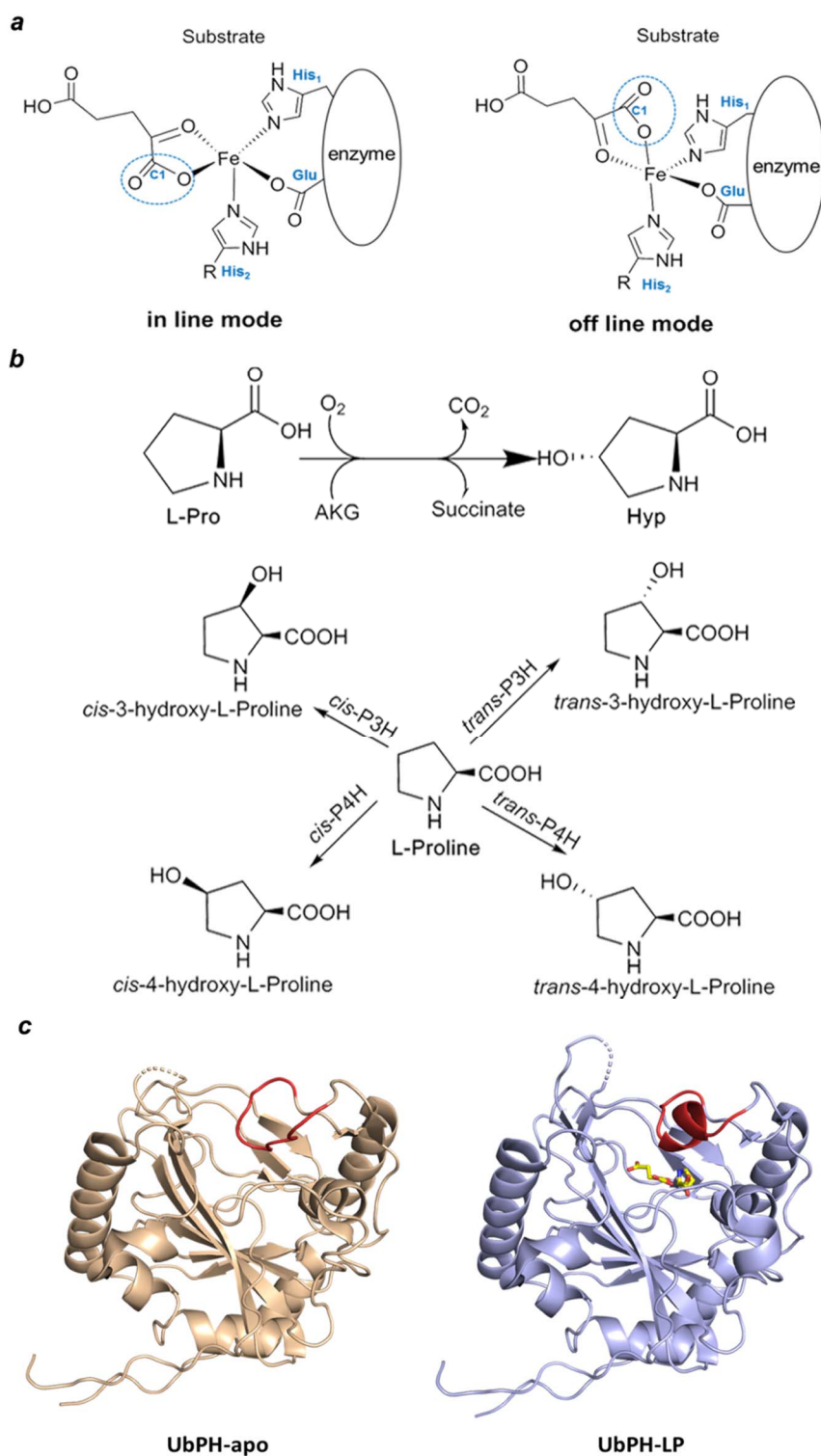


Figure S1 a. Chemical diagram of two types of AKG-dependent hydroxylase, in-line and off-line. In-line mode: AKG C-1 carboxylate opposite His₁. Off-line mode: AKG C-1 carboxylate opposite His₂. R=enzyme. b. Chemical transformation of L-proline to *trans*-4-hydroxy-L-proline catalyzed by UbPH and four hydroxylation forms of proline. Modified from references Hausinger, 2004 and Klein & Hüttel, 2011. c. The overall structures of UbPH-*apo* and UbPH-LP. The two subunits are colored in

wheat (UbPH-apo) and light blue (UbPH-LP) respectively. The flexible loop¹⁶⁸⁻¹⁷⁶ is shown in red.

UbPH-apo means ligand-free form (PDB code: 8H7T). UbPH-LP means AKG and L-proline binding form (PDB code: 8H7Y).

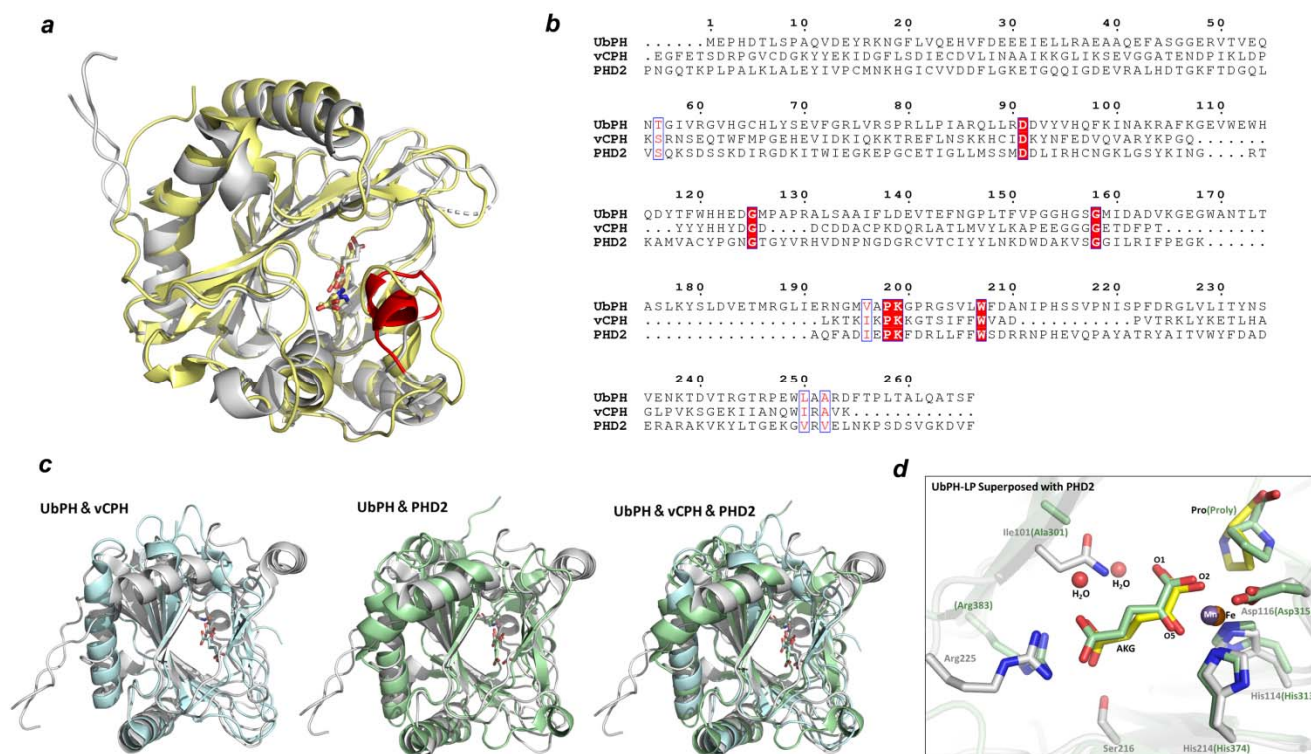


Figure S2 a. Superposed structure of UbPH (silver) and GriE (yellow, PDB code: 5NCI). The RMSD of the two structures' all corresponding C α atoms is 1.27 Å. The substrate binding loop 168-176 is shown in red. b. Sequence alignment of UbPH, vCPH (collagen Prolyl Hydroxylase, PDB code: 5C5T), and PHD2 (Hypoxia-Inducible Factor Prolyl Hydroxylase, PDB code: 5L9B). The same amino acids are in red block shown in white, and the similar amino acids are in white block and shown in red. c. Superposed structure of UbPH (silver) and vCPH (cyan, PDB code:5C5T). RMSD is 2.37Å. Superposed structure of UbPH (silver) and PHD2 (green, PDB code:5L9B). RMSD is 3.08Å. d. AKG binding site comparison of UbPH (silver) and Hypoxia-Inducible Factor Prolyl Hydroxylase (PHD2) (green, PDB code: 5L9B). Fe of UbPH is shown as an orange ball, Mn of PHD2 is shown as a purple ball, and water of PHD2 are shown as red balls and labeled. Residues are labeled and labels of PHD2 are shown in brackets.