

SUPPORTING INFORMATION

**Kinetic and Structural Analysis of Substrate Specificity in Two Copper**

**Amine Oxidases from *Hansenula polymorpha***

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**Table S1:** Comparison of  $k_{\text{cat}}$  Values in EDTA-Dialyzed vs Nondialyzed HPAO-2 Samples.

	<b>Benzylamine (150 <math>\mu\text{M}</math>)</b>	<b>Methylamine (5 mM)</b>
<b>HPAO-2 / (HPAO-2+EDTA)</b>	$1.9 \pm 1 \times 10^1 / 1.9 \pm 1 \times 10^1$	$1.69 \pm 0.07 / 1.9 \pm 0.1$

**Table S2:** Residue Changes that Define the O<sub>2</sub> Anteroom.

HPAO-2	HPAO-1
Ala406	Leu425
Phe595	Ile622
His612	Ile639
Val404	Ile423
Phe460	Leu440



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      410      420      430      440      450      460
HPAO-1 YWVFMQDGAIRLDIRLTGILNTYIILGDDEEAGPWGTRVYPNVNAHNHQHLFSLRIDPRID
      . : : : : . : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
HPAO-2 NIIFDQAGEIRIHVRATGILSTMPLE-DKDVTVPWGTNVGPRVMAAYHQHMLSFRIDPAVD
      400      410      420      430      * *

      470      480      490      500      510      520
HPAO-1 GDGNSAAACDAKSSPYPLGSPENMYGNAFYSEKTTFKTVKDSLNTYESATGRSWDIFNPN
      : : . . . : . : . . . : . . . : . . . : . . . : . . . : . . . : . . . :
HPAO-2 GYENTVVFFDDVIR--MEKNTKLNPNVNGFVTERTVVE--KPGYVEQSPFTNRSYKLIENEN
      450      460      470      480      490      500

      530      540      550      560      570      580
HPAO-1 KVNPIYSGKPPSYKLVSTQCPLLAKEGSLVAKRAPWASHSVNVVVPYKDNRLYPSGDHVPQ
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
HPAO-2 KINPIYSGKPPSYKLVSTQCPLLAKEGSLVAKRAPWASHSVNVVVPYKDNRLYPSGDHVPQ
      510      520      530      540      550      560

      590      600      610      620      630      640
HPAO-1 WSGDGVRRGMREWIGDGSSENIDNTDILFFHTFGITHFPAPEREDFPLMPAEPITLMLRPRHFF
      . : : : . : . : . : . : . : . : . : . : . : . : . : . : . : . : . :
HPAO-2 SQT--TGLGVW--ARRDENVRNDNPVWVWATLGFTHIPRVEDFPVMPVEAHEIALVDFGFF
      570      580      590      *600      610      620

      650      660      670      680      690
HPAO-1 TENPGLDIQPSYAMITSEAKRAVHKEETKDKTSRLAFEGSCCGK
      . : : : . : : : . : : : . : : : . : : : . : : : . : : : . : : :
HPAO-2 DKNPALSVPQS---TQSFNKSQYFPES-----GVESCCCKS
      630      640      650

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**Figure S2:** 10% SDS PAGE gel electrophoreses of HPAO-2

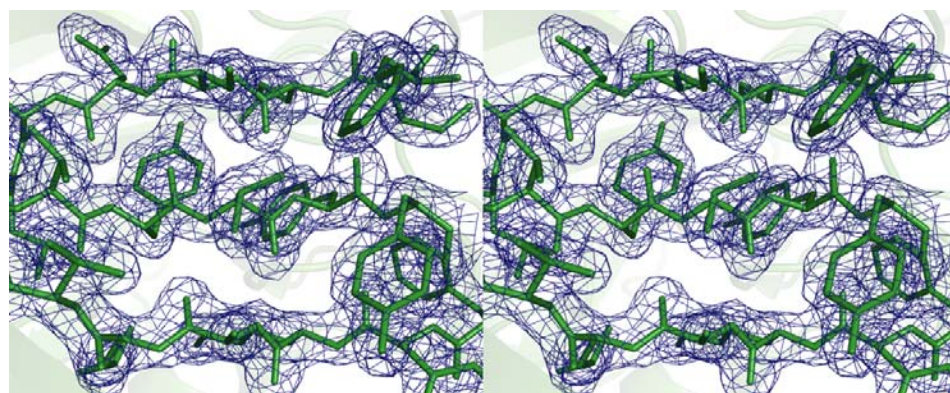
Lanes 1-4: 10  $\mu$ g protein loaded per lane. 1. Cell lysate, 2. Post Q-Sepharose, 3. Post S-300, 4. Bio-Rad Broad Range Ladder (150, 100, 75, 50, 37, 25, 20 kDa fragments visible.) Substantial purification of HPAO-2 is evident from the relative intensities of HPAO-2 to other minor contaminating bands in moving from lane 1 to lane 3.



**Figure S3:** Comparison of the DNA-derived N-terminal sequence of HPAO-2 and HPAO-1, including a peroxisomal signaling sequence (underlined) for HPAO-1, to the experimental N-terminal sequence for HPAO-2.

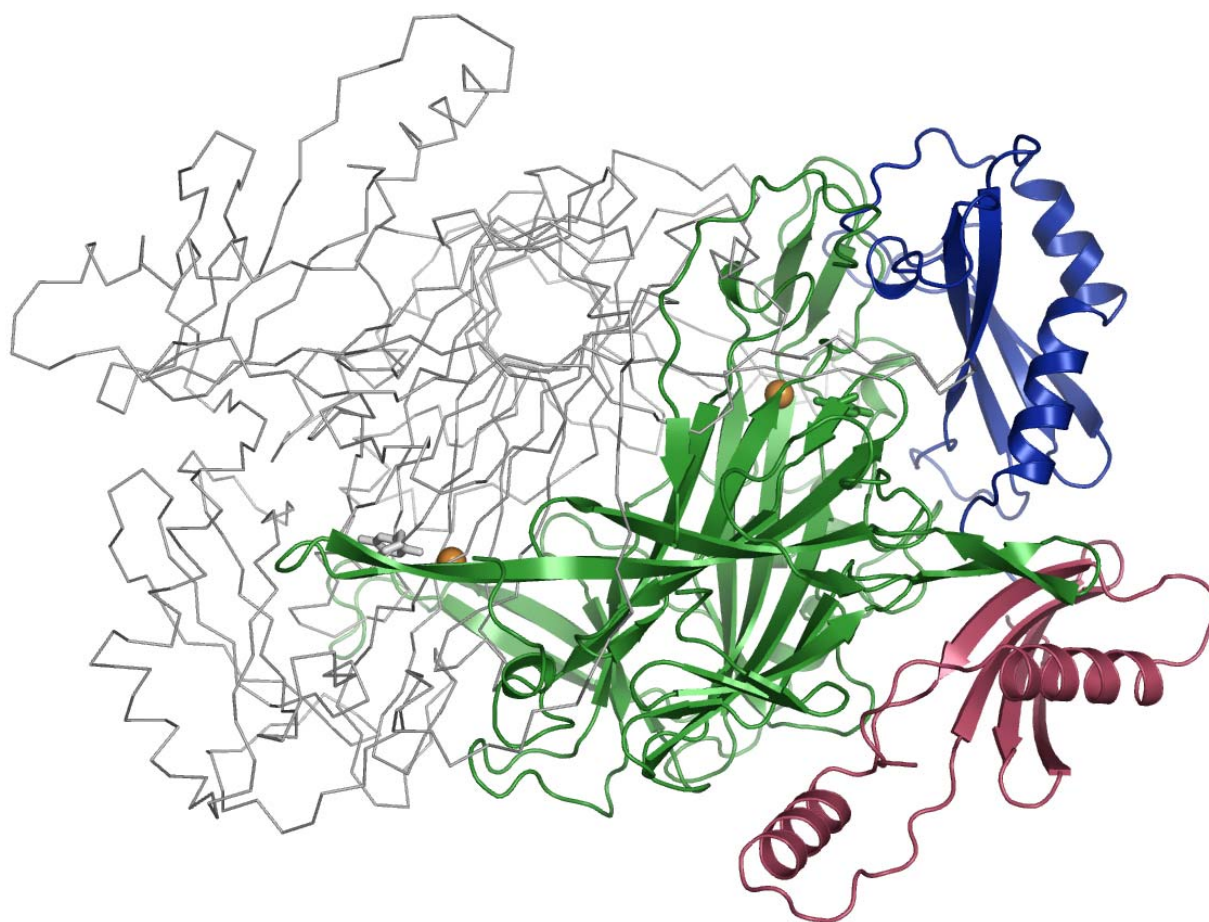
HPAO-1           MERLRQIASQATAASAAPARPAHPLDPLSTAEIKAA  
HPAO-2           -----MVHPYDPI SDAELQLT  
N-Term Seq:      -----VHPYDPI SDAELQLT

**Figure S4:** Stereo picture of the  $2F_o - F_c$  electron density quality (contoured at  $1.5\sigma$ ) within the HPAO-2  $\beta$ -sheet core. The model associated with the electron density is drawn explicitly in stick. The rest of the HPAO-2 model is drawn as green ribbon. Figure produced using PyMOL (<http://www.pymol.org/>).



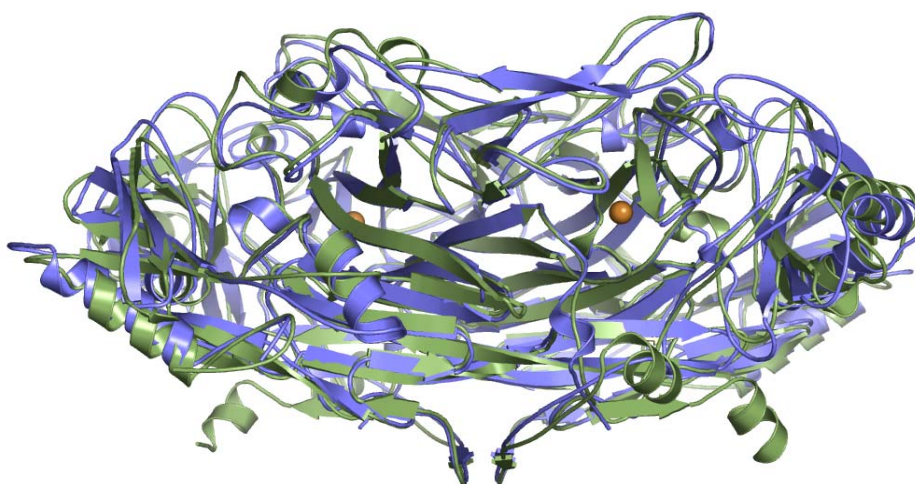


**Figure S5:** Overall fold of the HPAO-2 dimer. One monomer is displayed in ribbon and colored by domain from the N-terminus: D1 is shown in red, D2 in blue, and D3 in green. A second monomer is displayed in grey  $C_\alpha$  trace. TPQ is drawn in stick, and copper atoms are displayed as gold spheres. Figure produced using PyMOL (<http://www.pymol.org/>).

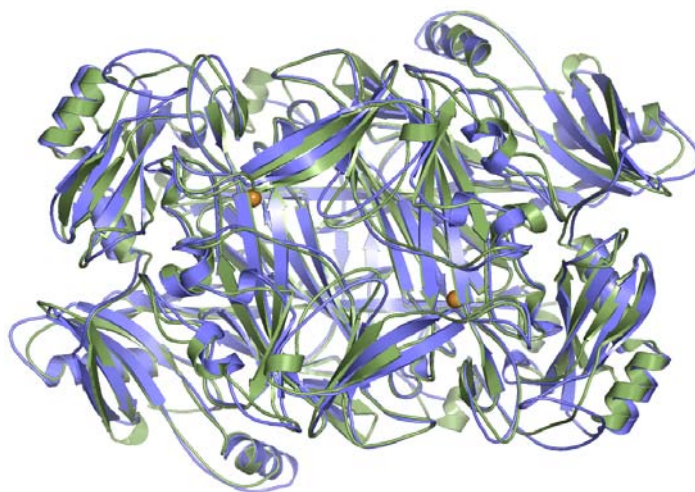


**Figure S6:** Ribbon diagrams showing an overlay of HPAO-1 and HPAO-2. HPAO-1 is colored blue, while HPAO-2 is colored green. Only the HPAO-2 copper ions (gold spheres) are displayed. **A.** View perpendicular to the molecular dyad axis. **B.** View along the molecular dyad axis by rotating the orientation in **A** forward by 90°. Figure produced using PyMOL (<http://www.pymol.org/>).

**A**

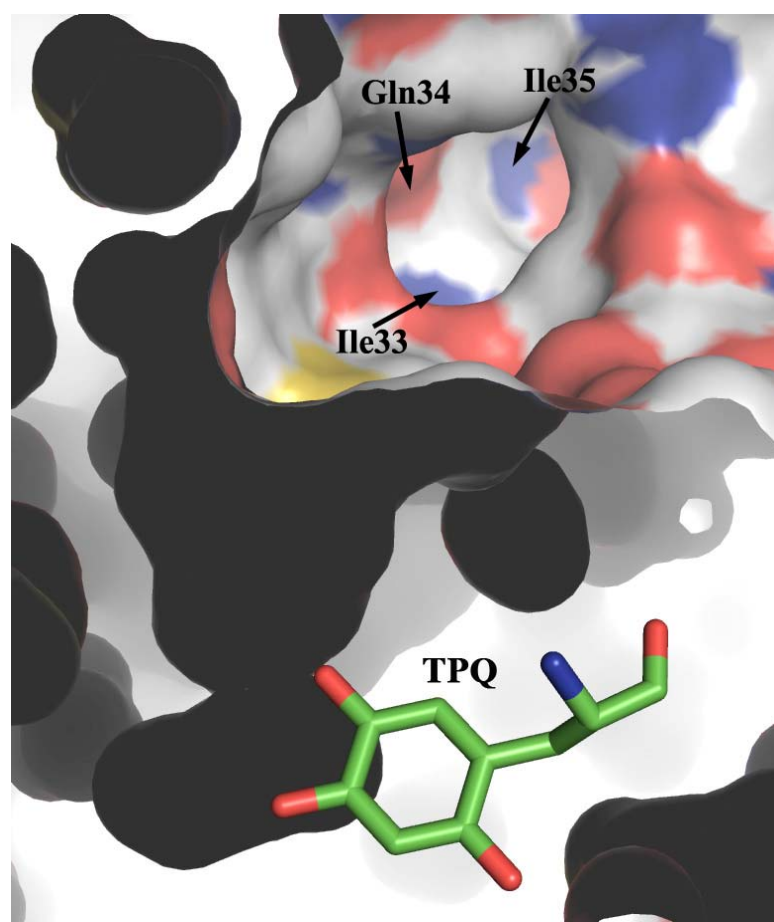


**B**

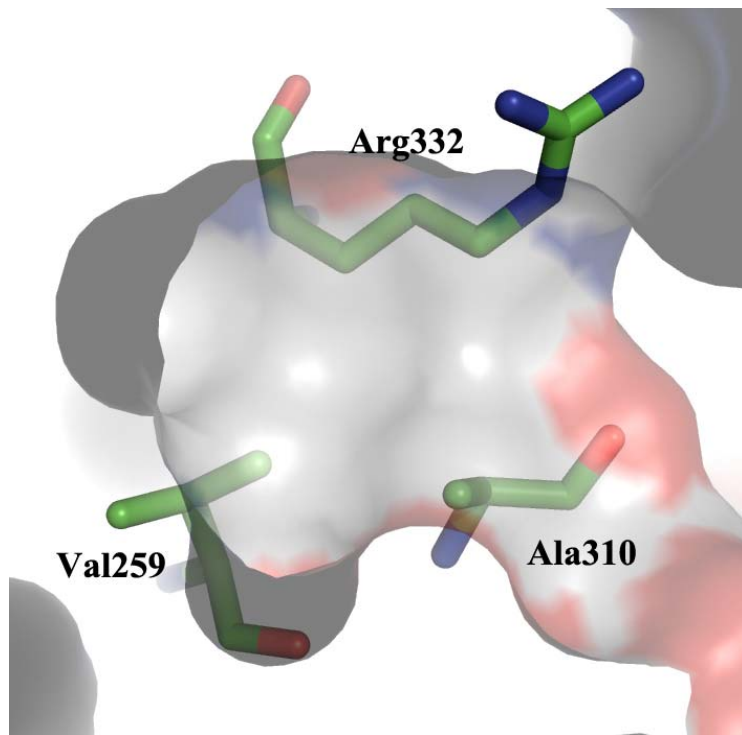


**Figure S7:** Surface representation of the side-chamber located in the HPAO-2 entry channel. **(A)** View looking into the side-chamber from the substrate entry channel. **(B)** Slice-through the side-chamber. The view in **B** is rotated about the vertical approximately 90° from the view in **A**. **(C)** Panel **B** with benzylamine modeled into the side-chamber. The protein surface is colored by atom type (carbon, light grey). TPQ and side-chains are drawn as stick colored by atom type (carbon, green). Surface is colored by atom type (carbon, grey). The model of benzylamine is drawn in stick colored by atom type (carbon, dark grey). Figure produced using PyMOL (<http://www.pymol.org/>).

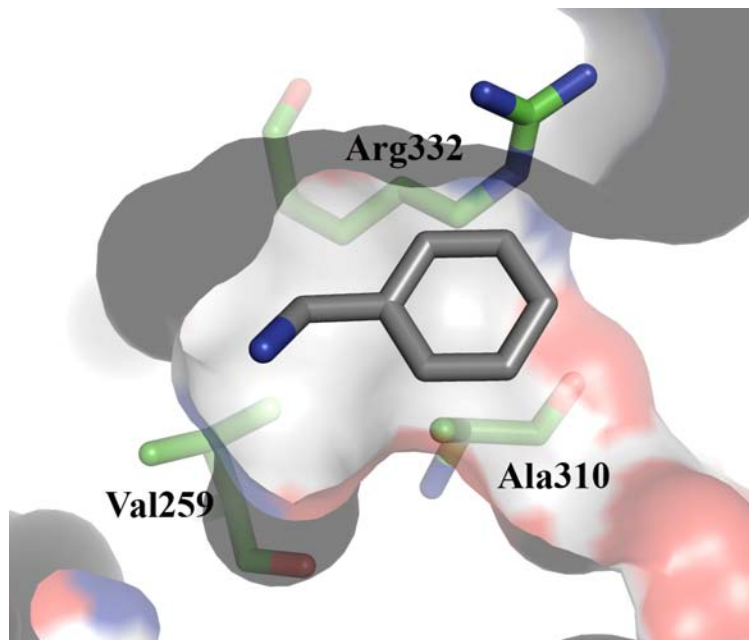
**A**



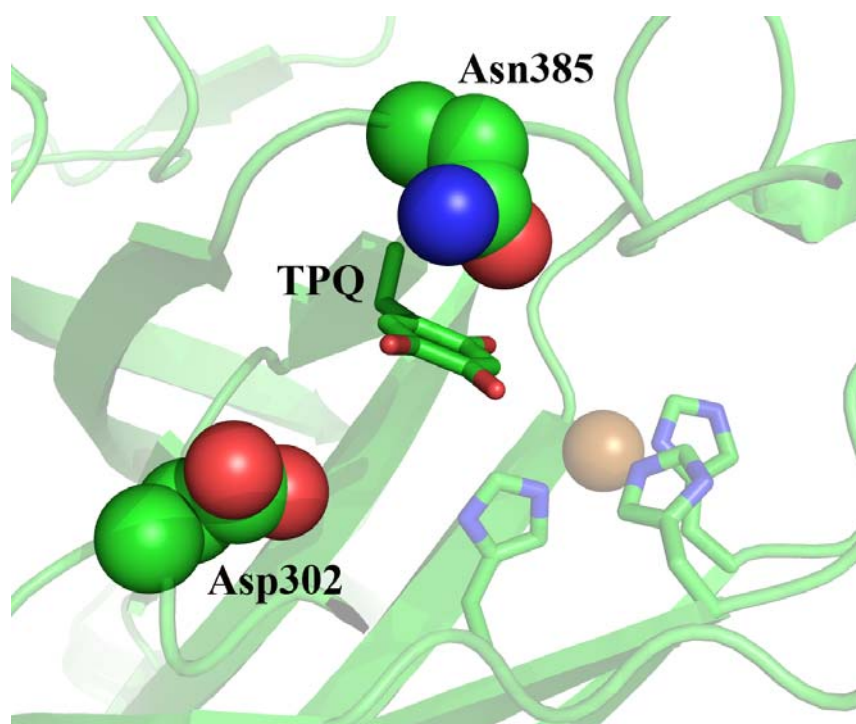
**B**



**C**



**Figure S8:** The active site of HPAO-2 showing the protein residues (Asp302 and Asn385) that lie either side of the TPQ ring. Protein residues are colored by atom type (carbon, green). Asp302 and Asn385 are drawn as space-filling spheres, TPQ and the histidines ligating the copper are drawn in stick, and the copper ion is displayed as a gold sphere. The fold of HPAO-2 is depicted in green ribbon. Figure produced using PyMOL (<http://www.pymol.org/>).



**Figure S9:** Model of the product Schiff base of benzylamine with TPQ. Residues from the crystal structures are drawn in stick colored by atom (carbon green, HPAO-2; carbon cyan, HPAO-1). The modeled product Schiff base is drawn in stick colored by atom (carbon dark grey). Figure produced using PyMOL (<http://www.pymol.org/>).

