Suppl.-Fig. 3
Legends to supplement-figures

**Supplement-Figure1:** Structurally related proteins to PPO. **a** Structural-Overlay of the enzymes most closely related to PPO (red) Monoamine-Oxidase (Binda et al., 2002) (MAO, blue) and Polyamine-Oxidase (Binda et al., 1999) (PAO, green). **b** Structure based multiple amino-acid-sequence-alignment of enzymes folding like p-Hydroxybenzoate-Hydroxylase (Schreuder et al., 1992) (PHBH): mitochondrial Protoporphyrinogen IX Oxidase from *N. tabacum* (PPO2_NT), human Monoamine-Oxidase B (MAO_B_HS), Polyamine-Oxidase from *Zea mays* (PAO_ZM), Phytoene-Desaturase (Norris et al., 1995) from *Arabidopsis thaliana* (PDS_AT) and D-Amino Acid Oxidase from *Sus scrofa* (Mizutani et al., 1996) (DAO_SS). Below the secondary structure elements (SSE) of PPO (in red and orange-yellow) and of MAO, PAO and DAO (in dark and light grey) are shown. Residues whose C\textsubscript{\alpha}-atoms deviate less than 1.0 Å from the C\textsubscript{\alpha}-atoms of the aligned residues of PPO are typed in blue and shaded in medium grey. These regions are all involved in FAD-binding as well as the region most strongly conserved in sequence (residues 15 to 65). So the high similarity in the structures can be explained by the FAD-binding-function of these enzymes. The substrate-binding domain and especially the membrane-binding domain of PPO are neither sequentially nor structurally related to the other enzymes, verified by a FASTA search on the swall protein sequence data base and the DALI-server (Holm and Sander, 1993).

**Supplement-Figure2:** The coordination of FAD in PPO2 resembles that of Polyamine Oxidase (Binda et al., 1999) (PAO) except for a neighbouring water molecule that is bound to N\textsubscript{5} of the FAD-isoalloxazine-moiety in PAO. This water-molecule might be responsible for hydrolysis of the product in the PAO-reaction, which does not occur in PPO.

**Supplement-Figure3:** Topology-diagram of the three domains of Protoporphyrinogen IX Oxidase from *N. tabacum* (PPO2NT). α-Helices are depicted as cylinders and β-strands as arrows. The FAD-binding domain is shown in red, binding regions of the cofactor FAD are painted in light grey, the substrate-binding domain is painted in green, the inhibitor INH
binding shown in medium grey and the membrane-binding domain (product channel) helices are shown in blue. Helices $\alpha_5$ and $\alpha_{14}$ form the dimer interface.

**Supplement-Figure 4:** a + b Molecular surfaces of PPO and HFc in the presumable docking regions coloured according to the electrostatic potential, positively charged patches are coloured in blue, negative ones in red and the uncharged and hydrophobic parts are shown in light grey. For mental reconstruction of the sandwich, lines and arrows are added.

a Bottom/Top view of the interacting surfaces. The PPO surface shows a hydrophobic patch at the binding interface. In the opening of the active site cavity FAD (red-orange), the inhibitor INH (green) and the Triton-X-100-molecule (blue) can be seen. The HFc-part of the channel is marked by a deep cavity into the enzyme marked by three Cholate-molecules (olive-green). The dimensions of the two channel-parts and the two-fold-axes in both enzymes fit together well. b Side view of the PPO-HFc interface after separating the complex by translating PPO upwards. The hydrophobic patch from PPO contacts the smaller lips of HFc and the border of the PPO-membrane-binding domain fits between the larger lips of HFc.


