STRUCTURAL CHARACTERIZATION OF OXYD, A CYTOCHROME P450 INVOLVED IN β-HYDROXYTYROSINE FORMATION IN VANCOMYCIN BIOSYNTHESIS*

SUPPLEMENTARY INFORMATION

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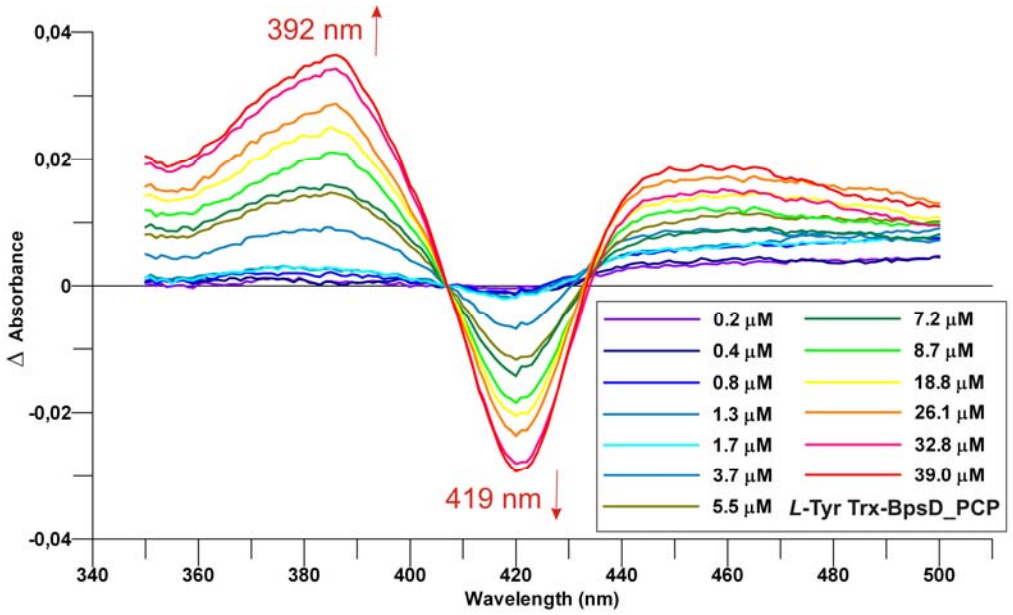


Supplementary Figure 1. Spectroscopic determination of the binding of (*L*)-tyrosine loaded Trx-BpsD_PCP to OxyD, measuring the effect on the haem iron environment due to ligand binding and concomitant water displacement.

Supplementary Table 1. Structural comparisons of known P450 structures with similarity to OxyD.

P450	PDB Entry	Percentage Sequence Identity (%)	RMSD (Cα, Å)	No. Aligned Residues/ Total Residues	*Z*-score (DALI)
Cyp125	3IW0	24	2.4	376/ 407	44.3
Cyp124	2WM4	27	2.4	377/ 426	43.6
Terp	1CPT	26	2.5	373/ 416	43.2
Cyp130	2WHF	27	2.6	375/ 394	43.0
BioI	3EJB	29	2.7	360/ 386	40.6
EryK	2WIO	27	2.8	366/ 393	40.5
EryF	1OXA	28	2.7	367/ 403	39.7
OxyC	1UED	24	2.9	368/ 391	38.9
Cyp154	1ODO	30	2.6	360/ 395	38.7
OxyB	1LFK	26	3.3	357/ 395	35.5

Supplementary Table 2. Alignment comparisons of known P450s with similar substrates to OxyD.

P450	Length	Sequence Identity (%)	Sequence Similarity (%)	PCP-Bound Substrate
NikQ	396	34	52	NikP1-(*L*)-Histidine
NovI	407	32	50	NovH-(*L*)-Tyrosine
CumD	407	33	48	CumC-(*L*)-Tyrosine
CloI	407	33	48	CloH-(*L*)-Tyrosine
SimD1	416	31	47	SimD6-(*L*)-Tyrosine
ZmbVIIc	416	35	52	ZmbVIIb-(*L*)-Valine
Ecm13	395	34	56	Ecm12-(*L*)-Tryptophan

Supplementary Table 3. Conserved sequence regions from alignment comparisons of known P450s with similar substrates to OxyD compared to closest matching P450 structures with known 3-dimeensonal structure.

P450	B-B_2 loop N-term.		B-B_2 loop C-term.						F-helix				G-helix					I-helix			β-1 sheet		
	69	72	82	83	84	86	89	90	171	172	173	174	187	188	189	190	193	228	229	235	282	283	284
Consensus (a)	**G**	L	A	(1)	G	**M**	V	T	H	**A**	W	S	**A**	(2)	N	**E**	(3)	N	**C**	**G**	(4)	L	**H**
BioI	P	-	V	Q	N	**M**	**F**	Q	S	L	**I**	D	M	A	V	Q	A	T	**C**	A	T	Q	M
OxyB	R	P	E	L	V	N	D	Y	-	-	-	-	L	G	D	K	R	F	**C**	A	P	**Y**	S
OxyC	-	-	Q	F	V	Q	T	Y	R	N	A	-	D	S	A	A	R	L	**C**	**G**	V	Q	A
Cyp124	N	**I**	E	Y	F	S	**V**	L	**V**	I	**L**	**G**	V	S	A	D	A	F	F	A	V	**V**	Y
Cyp125	**G**	P	V	Q	R	V	N	M	E	M	T	**G**	S	S	A	**E**	G	F	V	A	V	T	A
Cyp130	**G**	**V**	H	D	T	P	**M**	Q	A	I	**V**	**A**	**A**	V	G	S	A	F	T	**G**	V	Q	G
EryF	-	K	Y	F	A	N	T	**S**	E	I	**L**	-	**A**	A	R	**E**	N	I	A	A	P	E	T
EryK	-	-	**T**	-	P	**M**	E	I	A	L	**V**	D	V	L	**N**	P	A	F	S	A	F	P	Q
Cyp154A1	-	-	V	E	-	N	T	A	**L**	V	D	**G**	N	T	A	R	E	T	L	A	V	K	**H**
Terp	Y	**M**	I	-	-	S	S	M	D	F	**F**	**G**	T	I	A	T	D	Y	Y	A	V	K	S
Matches % (b)	20	30	10	0	0	20	30	10	20	0	60	50	20	0	10	20	0	0	30	20	0	20	10

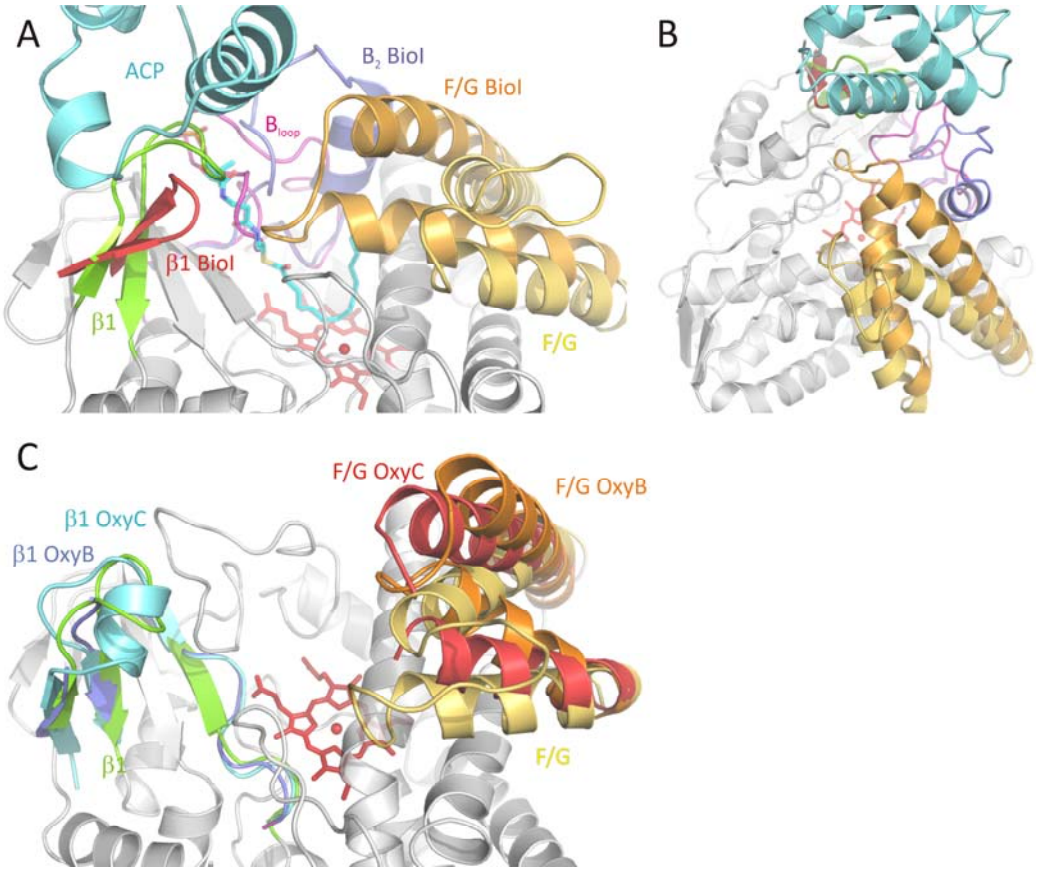
(a) Identity residues are emboldened and underlined, 1-2 mismatching residues or similar residues indicated in normal font. Exceptions are: (1) Small residue (S, G, A), (2) Positively Charged Residue (K, H, R), (3) Majority Hydrophobic (L, V; also S and G), (4) Majority Small (S, A; also V).

(b) Percentage of residues matching to the overall consensus rule, based upon similarity.

Supplementary Table 4. Alignment comparisons of individual NRPS PCP domains that interact with OxyD-like P450s and their similarity to BpsD_PCP.

P450	Region	Sequence Identity (%)	Sequence Similarity (%)	Alignment of Predicted Secondary Structure (%) [(a)]	PCP-Bound Substrate
NikP1	471-542	41	66	92	(*L*)-Histidine
NovH	524-600	35	52	92	(*L*)-Tyrosine
CumC	523-599	32	60	92	(*L*)-Tyrosine
CloH	524-600	35	77	92	(*L*)-Tyrosine
SimD6	907-997	40	64	92	(*L*)-Tyrosine
ZmbVIIb	510-572	40	64	95	(*L*)-Valine
Ecm12	525-598	40	60	92	(*L*)-Tryptophan
PCP-7S	962-1038	50	67	95	Vancomycin peptide

(a) Not including the terminus of helix α-4 due to differences in PCP length.



Supplementary Figure 2. OxyD structural comparisons. A and B – comparison of the major structural difference between the $P450_{BioI}$-ACP complex and OxyD (ACP and phosphopantetheine-linked fatty acid shown in cyan, OxyD B-B_2 loop shown in magenta, $P450_{BioI}$ B-B_2 loop shown in purple, OxyD partial β-1 sheet loop shown in green, $P450_{BioI}$ partial β-1 sheet shown in blue, OxyD F-G helices shown in yellow, $P450_{BioI}$ F-G helices shown in orange, OxyD protein shown in grey, heme shown in red); C – comparison of the major structural difference between the OxyB and OxyC phenolic coupling P450s and OxyD (OxyD partial β-1 sheet loop shown in green, OxyB partial β-1 sheet shown in blue, OxyC partial β-1 sheet shown in cyan, OxyD F-G helices shown in yellow, OxyB F-G helices shown in orange, OxyC F-G helices shown in red, OxyD protein shown in grey, heme shown in red, OxyD protein shown in grey, heme shown in red).

Structural Comparison to $P450_{BioI}$. Comparison of the OxyD structure to that of the acyl carrier protein (ACP) fatty acid bound $P450_{BioI}$ (CYP107H1) structure (RMSD 2.7 Å, PDB Code 3EJB (1)) at the broadest level indicates that the interaction of the amino acid bound PCP with OxyD is likely to be different to that seen in the $P450_{BioI}$-ACP complex. The first loop in the β-1 sheet of OxyD is extended away from the core of the protein, whereas in $P450_{BioI}$ a shortened loop in his region forms part of the interaction surface with the ACP-bound fatty acid (**Supplementary Figure 2A-B**). Additionally, the N-terminus of the A'-helix in OxyD now extends towards this longer β-1 loop. The B-B_2 loop, one of the substrate recognition sites (SRS) (2) in P450s, unsurprisingly exists in different conformations, with the B_2 helix missing in the OxyD structure and the OxyD loop not extending as far away vertically from the heme. The F-G helices form the upper surface of the active site and are displaced away from the B-B_2 loop region in the OxyD structure compared to the F-G helices in $P450_{BioI}$. The F-G helices themselves are shortened in OxyD whilst the F-G loop is comparatively longer, adopting a position similar to an open cap (**Supplementary Figure 2A-B**). An altered conformation of the SRS that interact with the ACP bound substrate in $P450_{BioI}$ would be expected in the case of the OxyD and its PCP binding partner, not only due to the difference in the carrier proteins but also in the nature of the substrates themselves (long, hydrophobic fatty acid vs. more hydrophilic, globular tyrosine).

Structural Comparison to Phenolic Coupling Oxy Proteins. Comparison of the structure of OxyD to the structures of OxyB (PDB Code 1LFK (3)) and OxyC (PDB Code 1UED (4)), the PCP-heptapeptide phenolic coupling P450s from vancomycin biosynthesis, indicate significant differences in active site structure and a relatively large RMSD (3.3 Å and 2.9 Å respectively) (**Supplementary Figure 2C**), although care must be taken due to the presence of different molecules within the active sites of these three P450s (OxyB: empty, OxyC: PEG-bound; OxyD: glycerol-bound). Specifically, the β-strand forming one side of the active site is different in the peptide phenolic coupling Oxy proteins, with a Pro-Thr/Asn-Pro kink forcing the β-sheet to run across the heme propionate group, whereas in OxyD the Pro-Thr/Asn-Pro residues are replaced by Val-Leu, restoring a typical β-sheet structure. The geometry of the I-helix at the acid/alcohol pair in OxyD is altered in a manner such that these residues project further into the active site above the heme. The F-G helices in OxyD

are again moved away from the B-B_2 loop region and closer to the I-helix, thus producing a smaller active site directly above the heme, but one more open towards the B-B_2 region than that found in OxyB and OxyC (**Supplementary Figure 2C**). The C-terminal loop that projects into the active site of all the proteins is shifted towards the A-helix in the OxyD structure due to hydrogen bond from His-284 to Ile-381. A small α-helical section in the β-1 loop extension of OxyC is an additional difference to the OxyD structure, likely due to the surface interactions required for PCP binding and presentation of their very different substrates.

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