X-ray Structure of a Hydroxylase-Regulatory Protein Complex from a Hydrocarbon-Oxidizing Multicomponent Monoxygenase, *Pseudomonas stutzeri* OX1 Phenol Hydroxylase^{†,‡}

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Running Title: Structure of a Phenol Hydroxylase-Regulatory Protein Complex

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^{*} The coordinates and structure factors for the PHH-PHM complex have been deposited in the Protein Data Bank (entries XXXX and XXXX) for the native and SeMet enzyme, respectively.

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Figure S1. Structure and electrostatic surfaces of (A) PHH, (B) ToMOH, and (C) MMOH. The hydroxylase α , β , and γ subunits are colored gray, blue, and purple, respectively. Protein and electrostatic surfaces of (D) PHM, and of those predicted by homology for (E) T4MOD and (F) MMOB, that pack against the hydroxylase are also shown. Electrostatic surfaces were generated by using APBS in PyMol. Red surfaces are negatively charged and blue surfaces are positively charged.



Figure S2. Structures of the (A) PHH and (B) ToMOH γ -subunits. The α -helices and β -strands are labeled accordingly.



Figure S3. (A) Interaction of the PHH β -subunit N-terminus (purple) with α -subunit helices E and F (gray) on the hydroxylase surface in which PHM is not bound. (B) Packing interaction of PHH and PHM molecules in the crystal lattice. The γ -subunit (green) of one PHH molecule (blue) packs against the canyon region of an adjacent PHH molecule (purple). PHM is colored red.



Figure S4. Structural comparisons between BMM regulatory proteins. Alignments of (A) PHM (red) and MMOH (purple) and (B) PHM and T4MOD (green) in stereo.

		β1β2	>
РН	PHM DmpM	MSQLVFIVFQDNDDSRYLAEAVMEDNPDAEMQ	2H 33 2H 33
тмо	T4MOD ToMOD AMOD	RSTLADQALHNNNVGPIIRAGDLVEPVIETAEIDNPGKEITVE MTTNTVQTLSASDNALNNNMVGPVLRAGDVAIAVGEAAEIDNPGKEIKVI 	ED 44 DD 51 IE 44
SsoMO	SsOMOD	MSLELTLETILDKYQIMDLSKLPQNMVGPVLMKDEFSYAVVEALARDNPNTFKGVI	LD 57
sMMO	MMOB-Mc MMOB-Mt BMOB	MSVNS <mark>NAY</mark> D <mark>AG</mark> IMGLK <mark>G</mark> KDFADQFFADENQVVHESDTVVLVLKKSD <mark>E</mark> INTFIEEILLTDYKKNVNPTVNVE MSSAHNAYNAGIMQKTGKAFADEFFAEENQVV <mark>H</mark> ES <mark>NAVVLVLMKSD</mark> EID <mark>AI</mark> IEDIVLKG-GKAKNPSIVVE MS-NV <mark>NAY</mark> HAGTNGKE <mark>G</mark> QDFIDDFLSEENSALPTSEAVVLALMKTEEIDAVVDEMIKPQMEDNPTIAVE	3D 72 3D 71 3D 69
AMO	AMOB-Nor AMOB-Mr	PDAKI MSTRDFTKVRDTVGISLIGSAETAETVAMVEEEIPDAKI MSSPTPARDRTQVRDTVGISLIGSSETSVIVDMVAELVPDAKI	FD 41 FD 45
THFMC	PMOB-Gor THFMOB	BOFGADTEFSNMCGVTLMNTPIGRVVADVMGAKDGVEL MTDATATSAAENGSGDAAYDYVGLVMQRTPEGEAVGRVCSQTPGVEVI	FE 40 FT 49
		$-\underline{\beta}_{3} - \underline{\beta}_{4} - \underline{\alpha}_{2} - \underline{\alpha}_{3} - \underline{\beta}_{5} - \underline{\beta}_{6} - \underline{\beta}_{7} - \underline{\beta}_{7}$	
PH	PHM DmpM	QPAMIRIQAEKRLVINRETMEEKL <mark>G</mark> RDWDVQEMLINVI <mark>S</mark> IAGNVDEDDDHFILEWN	89 90
тмо	T4MOD ToMOD AMOD	RRAYVRIAAEGELILTRKTLEEQL <mark>G</mark> RPFNMQELEINLA <mark>S</mark> FAGQIQADEDQIRFYFDKTM KLAYVRIGAEDELILRKETIEECLGRPFRMQELEINLSSFAGIIDMDFDRVRFYFNKHL RGDYVRIHTDRDCRLTRASIEQALGRSFVLAAIEAEMS <mark>S</mark> FKGRMSSSDSEMRWYYKS	103 110 101
SsoMO	SsOMOD	RGSYIRVVGERELILNKTTLEEVI <mark>G</mark> MDVRFPGEVEVRM <mark>S</mark> AFAGKIIVRGDYIKWHLDL	115
sMMO	MMOB-Mc MMOB-Mt BMOB	RAGYW <mark>W</mark> IKANGKIEVDCDEISEL <mark>LGRQF</mark> NVYDFLV <mark>D</mark> VS <mark>STIGRAY</mark> TLGNKFTITSELM <mark>GL</mark> DRKLEDYHA K <mark>AG</mark> FWWIKADGAIEIDAAEAGELLGKPFS <mark>W</mark> Y <mark>DLLINV</mark> SST <mark>WGRA</mark> Y <mark>TL</mark> GTK <mark>W</mark> TITSELMGLDRALTDI RGGYWWIKANGKIVIDCDEATELL <mark>G</mark> KKYTVYDLLVNVS <mark>T</mark> TVGRAMTLGNQFIITNELL <mark>GL</mark> ETKVESVY	141 138 137
AMO	AMOB-Nor AMOB-Mr	NDCFYKIEREGLLRFDMENLSERL <mark>G</mark> RPYSVHDFLVNMT <mark>S</mark> YYGRIVVNDGVIEIHTEILPDRFRD NDVFFKIERDGMLSFDMVELSDRL <mark>G</mark> KPYTVHDFLVNMT <mark>S</mark> YYGRIVVKDHGIEIHSEILPERFRD	105 109

Figure S5. Sequence alignment of BMM regulatory proteins within their specific subfamilies. PHM residues highlighted in gray form contacts with the PHH surface. Residues in green mark MMOB positions that experience greater than average NMR line broadening in the presence of MMOH. Teal shading denotes highly conserved sMMOH residues in the N- and C-termini that may be important for function. Blue shading denotes a strictly conserved residue while red shading marks a conserved residue having important catalytic function. The regulatory protein sequences and accession numbers used for the alignment are: PHM, *Pseudomonas stutzeri* OX1 (AAO47357), DmpM *Pseudomonas* sp. strain CF600 (P19731); T4MOD, *Pseudomonas mendocina* (2BF2_B); ToMOD, *Pseudomonas stutzeri* OX1 (AAT40434); AMOD, Xanthobacter autotrophicus Py2 (CAA09914); SsoMOD, Sulfolobus solfataricus P2 (NP_342687); MMOB-Mc, Methylococcus capsulatus Bath (P18797); MMOB-Mt, Methylosinus trichosporium OB3b (P27356); BMOB, Pseudomonas butanovora (AAM19729); AMOB-Nor, Nocardioides sp. JS614 (AAV52083); AMOB-Mr, Mycobacterium rhodesiae (AAO48575); PMOB, Gordonia sp. TY-5 (BAD03959), THFMOB, Pseudonocardia sp. K1 (CAC10510).



Figure S6. Stereo view of the $|2F_o| - |F_c|$ simulated-annealing omit electron density maps (green) surrounding the native PHH diiron active sites from (A) chain A and (B) PHM-bound chain B contoured to 1.2 σ .

sMMO	IYIDRVSQVP-FCPSLAKGASTLRVHEYNGQMHTFSDQWGERM	456
AMO	P-MCQVCQVPCVMPRLDMNAARIIEFEGQKIALCSEPCQRI	430
PhN	PQL <mark>C</mark> QV <mark>C</mark> QIPTIFTEKD-APTMLSHRQIEHEGERYHF <mark>C</mark> SDG <mark>C</mark> CDI	439
MopN	PQLCQVCQIPMTFFEMDGDPTLFSYRDSIYKDERYHTCSDGCHDI	447
PhhN	PHLCQVCQVPAIFFEPD-DPTKLSLRSLVHEGERYHFCSDGCCDI	439
PhlD	PHLCQVCQVPAIFFEPD-DPTKLSLRSLVHEGERYHFCSDGCCDI	439
DmpN	PHLCQVCQLPVIFFEPD-DPTKLSLRSLVHEGERYQFCSDGCCDI	440
PhcN	PMLCTTCQIPMGFFEP-GDATKICYRESDYEGSKYHFCSDGCKHV	441
TbmD	PMLCTTCQIPMGFFEP-GDATKIAYRESDYFGMKYHFCSDHCKHI	440
TomA	PMLCTTCQIPMIFFEP-GDATKICYRESAYLGDKYHFCSDHCKEI	441
PoxD	PML <mark>C</mark> TT <mark>C</mark> QIPMIFFEPD-DPTQTCYRESSYHGMKFHF <mark>C</mark> SDG <mark>C</mark> KDI	438

TOMO PTICNMCNLPIAHT--PGNKWNVKDYQLEYEGRLYHFGSEADRWC 435

Figure S7. Sequence alignment of the C-terminal portion of the PH hydroxylase αsubunit depicting the conserved cysteines contributing to the zinc binding site in PHH. The alignment was adapted from Leahy, et al. (3). Phenol hydroxylase α-subunit sequences are from *Pseudomonas stutzeri* OX1 (PhN), *Acinetobacter calcoaceticus* NCIB8250 (MopN), *Pseudomonas putida* P35X (PhhN), *Pseudomonas putida* H (PhID), *Pseudomonas* sp. Strain CF600 (DmpN), *Comanonas testosteroni* R5 (PhcN), *Burkholderia cepacia* JS150 (TbmD), *Burkholderia cepacia* G4 (TomA3), and *Ralstonia eutropha* E2 (PoxD).



Figure S8. Structural changes in helix F. A $|2F_o| - |F_c|$ simulated annealing electron density omit map (red) contoured at 1.0 σ around α -subunit helices E and F from the SeMet PHH-PHM structure (orange), depicts structural changes in stereo. Residues in green depict the unaltered conformation of helix E and F residues in the other PHH α -subunits.