Supporting Information

High Resolution XFEL Structure of the Soluble Methane Monooxygenase Hydroxylase Complex with its Regulatory Component at Ambient Temperature in Two Oxidation States

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SUPPLEMENTAL RESULTS

Table S1. X-ray Data Collection and Refinement Statistics for MMOH:MMOB Complex in the Diferric, Diferrous, Reoxidized States, and the t = 0 Diferrous State Before O₂ Exposure.

Dataset (PDB Code)	Diferric MMOH:MMOB (PDB 6YD0)	Diferrous MMOH:MMOB (PDB 6YDI)	Reoxidized MMOH:MMOB (PDB 6YDU)	t = 0 Diferrous MMOH:MMOB (PDB 6YY3)
Wavelength	1.30644	1.30448	1.30396	1.318066
XFEL source	LCLS MFX	LCLS MFX	LCLS MFX	PAL-XFEL NCI
Spacegroup	P41212	P41212	P41212	P41212
Cell dimensions (Å)	106.9, 106.9, 303.9	106.9, 106.9, 303.9	106.9, 106.9, 304.1	106.0, 106.0, 301.0
Cell angles (deg)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution range ^{<i>a</i>} (Å)	33.00-1.95 (2.02- 1.95)	33.71-1.95 (2.02- 1.95)	33.96-1.95 (2.02- 1.95)	24.66-2.0 (2.07- 2.0)
Merged lattices	25274	14980	15629	13596
Reflections observed (unique) ^b	16675375 (128780)	10382513 (128981)	9590816 (128969)	9549089 (116482)
Mean $\langle I \rangle / \sigma \langle I \rangle^{a,b,c}$	34.3 (0.8)	27.5 (0.6)	25.2 (0.6)	1.78 (0.32)
CC1/2 ^{<i>a,b</i>}	0.944 (0.282)	0.949 (0.175)	0.945 (0.077)	0.933 (0.25)
Completeness (%) ^{<i>a.b</i>}	99.93 (99.90)	99.91 (99.70)	99.84 (99.16)	99.06 (92.35)
$R, R_{free}, \text{test } (\%)^d$	16.24, 18.96, 1.55	17.54, 19.54, 1.55	16.77, 19.64, 1.55	20.36, 23.50, 1.71
R_{split} (%)	20.8 (100.6)	20.6 (110.8)	21.6 (119.8)	24.1 (90.8)
RMSD ^{<i>e</i>} bond length (Å)	0.017	0.004	0.010	0.007
RMSD ^{<i>e</i>} angles (deg)	1.33	0.72	0.99	0.81
Average B-factor	45.40	46.07	46.51	45.36
Ramachandran favored, allowed and outlier regions (%)	96.50, 3.33, 0.17	97.42, 2.58, 0.00	97.09, 2.91, 0.00	97.17, 2.83, 0.00

^{*a*} Values for the highest resolution shell are given in parentheses. ^{*b*}These statistics are for merging the data in P4₁. The data were subsequently merged into P4₁2₁2, essentially halving the number of unique reflections and improving the completeness, $\langle I \rangle / \sigma \langle I \rangle$, and CC1/2 beyond what is reported here. ^{*c*}Columns 1-3: σ was computed using the Ha14 error model as described in Hattne, et al.¹ and Brewster et al. 2018.² Column 4: σ was computed using the Ev11 error model (see Brewster et al. 2018² and 2019³). The difference in error models accounts for the large difference in $\langle I \rangle / \sigma \langle I \rangle$ values when comparing columns 1-3 with column 4; see Brewster et al.³ for more information. ^{*d*} $R = (\sum |F_{obs}-kF_{calc}|)/\sum |F_{obs}|$, where k is a scale factor. The R_{free} value was calculated with the indicated percentage of reflections not used in the refinement. ^{*e*} Root-mean-square deviation (RMSD) from ideal geometry in the final models.

Table S2. List of MMOH (α-Subunit) Residues that Undergo a Large Structural Rearrangement upon MMOB Binding.^{*a*}

Position	Residues reorganized	Specific interaction with MMOB
Loop 2	A53, N54, E55, T56, K57, E58, Q59, F60, K61, V62	K57 hydrogen bond to D137 Q59 hydrogen bond to A115(b) K61 hydrogen bond to T136(b)
Helix A	R69	R69 hydrogen bond to D103
Helix A	R77	R77 hydrogen bond to E47
Helix B	L110	L110 steric clash with L216 (MMOH)
Helix E	L204, Q205, L206, V207, G208, E209, A210, C211, F212, T213, N214, P215, L216, I217, V218, A219, V220, T221, E222, W223	N214 hydrogen-bond to S110 V218 steric clash with F75
Helix F	F236, L237, S238, V239, E240, T241, D242, E243, L244, R245, H246, M247, A248, N249, G250, Y251, Q252	L237 hydrogen bond to S109(b) E240 steric clash with S109 L244 steric clash with T111 Y251 steric clash with L128
Loop 9	S256, I257, A258, N259, D260, P261, A262, S263	
Helix G	W276, F282, T283, L286, F290	
Loop 10	V298, E299	E299 hydrogen bond to Y8
Helix H, Loop 11 and Helix 4	V302, K303, T304, W305, N306, R307, W308, V309, Y310, E311, D312, W313, G314, G315, I316, W317, I318, G319, R320, L321, G322, K323, Y324, G325, V326, E327, S328, P329, A330, S331, L332, R333, D334, A335, K336, R337, D338, A339, Y340, W341, A342, H343	V302 steric clash with F20 K303 steric clash with N9 and M13 K303 hydrogen bond to L15(b) N306 steric clash with F24 R307 steric clash with Y8 W308 steric clash with P215 (MMOH) Y310 hydrogen bond to V31(b) D312 hydrogen bond to K79 I316 steric clash with S35 R320 steric clash with R114 R320 hydrogen bond to S126(b) K323 steric clash with L132 Y324 steric clash with L131 Y324 hydrogen bond to D132 R333 steric clash with Q30 R333 hydrogen bond with E27 K336 hydrogen bond to F24(b) and N29(b)

^{*a*} The 1MHY and the 6YD0 structure have been compared in order to generate this table. The specific interactions between MMOH and MMOB residues are listed in the right-most column. Some MMOH residues interact with the main-chain atoms in MMOB and these have been indicated with the (b) symbol. Some MMOH residues reorganize as an indirect effect of MMOB binding for they interact with MMOH residues that rearrange as a result of direct interaction with MMOB.

Table S3. List of Hydrogen Bond Donor-Acceptor Distances (in Angstroms) in MMOHAlone (1MHY) and the Diferric (6YD0) and Diferrous Forms (6YDI) of the MMOH:MMOBComplex.

Donor Acceptor pair	1MHY	6YD0	6YDI
D242-H147	2.67	2.60	2.66
D242-R146	2.92	2.86	2.81
D242-E71	2.80	2.60	2.66
D242-K74	2.78	2.71	2.69
D143-H246	2.76	2.75	2.83
D143-R245	2.95	2.76	2.81
D143-Y67	2.68	2.62	2.61

	10A	20F	30Q	40L	50A	60G	69V
BAE86877.1	MSLSSNAYGAG	MAKSGKE FADEY F	AEENQVVHE S	NEVVLVLKKS	SDEINIIVDEI	LLGDRKD-EN	PTLVVE
WP_017840377.1	MSLSSNAYGAG	MAKSGKE <mark>F</mark> ADEY <mark>F</mark>	AEEN <mark>Q</mark> VVHE S	NEVVLVLKKS	S DE I N I V V DE I	LLGERKD-EN	PTLVVE
WP_013818323.1	MSKSSNAYNAG	MQKNGKA <mark>F</mark> ADEY <mark>F</mark>	SEDN <mark>Q</mark> VVHE S	HEVVLVLKKS	S D E L N T V V E E I	LLGSHKA-DN	PTLVVE
AAF04158.2	MSVNSNAYDAG	MGL KGKD <mark>F</mark> ADQ F <mark>F</mark>	ADEN <mark>Q</mark> VVHE S	DTVVLVLKKS	SDEINTFIEEI	LLTDYKKNVN	PTVNVE
pdbl2MOBl	MSSAHNAYNAG	MQKTGKA <mark>F</mark> ADEF <mark>F</mark>	AEEN <mark>Q</mark> VVHE S	NAVVLVLMKS	SDEIDAIIEDI	VLKGGKA-KN	PSIVVE
AAC45291.1	MSSAHNAYNAG	MQKTGKA <mark>F</mark> ADEF <mark>F</mark>	AEEN <mark>Q</mark> VVHE S	NAVVLVLMKS	S D E I D A I I E E M	VLKGGKA-KN	PSIVVE
WP_102844999.1	- MT AKNAYNAG	MKKSGEA <mark>F</mark> AAEF <mark>F</mark>	AEEN <mark>Q</mark> VVHE S	NTVVLVLMKS	SDEIDAIVEEL	IMGEEAK - RN	PTLVVE
VFU08594.1	- MT AKNAYNAG	MKKSGEA <mark>F</mark> AAEF <mark>F</mark>	AEEN <mark>Q</mark> VVHE S	NTVVLVLMKS	SDEIDAIVEDI	ILGEETK-RN	PTLVVE
WP_020174571.1	- MT AR NAYNAG	MKKNGE A <mark>F</mark> AKE F <mark>F</mark>	AEEN <mark>Q</mark> VVHE S	NTVVLVLMKS	SDEIDAIVEDI	ILGEEAK-RN	PTLVVE
KJB91174.1	- MM S K N S Y G A G	MAKT GE A <mark>F</mark> A R E F <mark>F</mark>	A E E N <mark>Q</mark> V V K E A	NKVVLVLMKS	SDEVDAIVEEM	ILKDGII-RN	PTMTVE
	701	80 4	OOF	1005	110C	12014	. 1
	79K	89A	99F	109S	1,19G	129M	+1
BAE86877.1	79K DRAGYWWLKATO	89A G K I E V D C E E V S E L L	99F GRTFSVYDFL	109S VDV <mark>SST</mark> IGRA	119G AFTLGEKFTIT	129M SELMGLDRKL	+1 E D L K A A
BAE86877.1 WP_017840377.1	79K DRAGYWWLKATC DRAGYWWLKATC	89A 3 K I E V D C E E V S E L L 3 K I E V D C E E V S E L L	99F GRTFSVYDFL GRTFSVYDFL	109S VDV <mark>SST</mark> IGRA VDV <mark>SST</mark> IGRA	1,19G A FTL GEK FT I T A YTL GEK FT I T	129M SELMGLDRKL SELMGLDRDL	+1 E D L K A A E D L K A A
BAE86877.1 WP_017840377.1 WP_013818323.1	79K DRAGYWWLKATC DRAGYWWLKATC DRAGFWWLKATC	89A G K I E V D C E E V S E L L G K I E V D C E E V S E L L G K I E I D C V E V S E L L	99F GRTFSVYDFL GRTFSVYDFL GKHYSVYDLL	109S VDV <mark>SST</mark> IGRA VDVSSTIGRA VDVSSTIGRA	1,19G A F T L G E K F T I T A Y T L G E K F T I T A Y T L G E T F T I T	129M SELMGLDRKL SELMGLDRDL SELMGLDVKL	+1 E D L K A A E D L K A A K D L A A A
BAE86877.1 WP_017840377.1 WP_013818323.1 AAF04158.2	79K DRAGYWWLKATC DRAGYWWLKATC DRAGFWWLKATC DRAGYWWIKANC	89A GKIEVDCEEVSELL GKIEVDCEEVSELL GKIEIDCVEVSELL GKIEVDCDEISELL	99F GRTFSVYDFL GRTFSVYDFL GKHYSVYDLL GRQFNVYDFL	109S VDVSSTIGRA VDVSSTIGRA VDVSSTIGRA VDVSSTIGRA	l 19G AFTLGEKFTIT AYTLGEKFTIT AYTLGETFTIT AYTLGNKFTIT	129M SELMGLDRKL SELMGLDRDL SELMGLDVKL SELMGLDRKL	+1 E DL KAA E DL KAA KDL AAA E DYHA -
BAE86877.1 WP_017840377.1 WP_013818323.1 AAF04158.2 pdbl2MOBl	79K DRAGYWWLKATC DRAGYWWLKATC DRAGFWWLKATC DRAGYWWIKANC DKAGFWWIKADC	89A GKIEVDCEEVSELL GKIEVDCEEVSELL GKIEIDCVEVSELL GAIEIDAAEAGELL	99F GRT FS VYD FL GRT FS VYD FL GKHY S VYD LL GRQ FN VYD FL GKP FS VYD LL	109S VDVSSTIGRA VDVSSTIGRA VDVSSTIGRA VDVSSTIGRA INVSSTVGRA	LIPG AFTLGEKFTIT AYTLGEKFTIT AYTLGETFTIT AYTLGNKFTIT AYTLGTKFTIT	129M SELMGLDRKL SELMGLDRDL SELMGLDVKL SELMGLDRKL SELMGLDRAL	+1 E DL K A A E DL K A A K DL A A A E D Y H A - T D I
BAE86877.1 WP_017840377.1 WP_013818323.1 AAF04158.2 pdbl2MOBI AAC45291.1	79K DRAGYWWLKATC DRAGYWWLKATC DRAGFWWLKATC DRAGYWWIKANC DKAGFWWIKADC DKAGFWWIKADC	89A GKIEVDCEEVSELL GKIEVDCEEVSELL GKIEIDCVEVSELL GKIEVDCDEISELL GAIEIDAAEAGELL GAIEIDAAEAADLL	99F GRTFSVYDFL GRTFSVYDFL GKHYSVYDLL GRQFNVYDFL GKPFSVYDLL GKPFSVYDLL	109S VDVSSTIGRA VDVSSTIGRA VDVSSTIGRA VDVSSTIGRA INVSSTVGRA VNVSSTVGRA	119G A FTL GE K FT I T A YTL GE K FT I T A YTL GE T FT I T A YTL GT K FT I T A YTL GT K FT I T A YTL GT K FT I T	129M SELMGLDRKL SELMGLDRVL SELMGLDVKL SELMGLDRKL SELMGLDRAL	+1 E DL K A A E DL K A A K DL A A A E D Y H A - T D I T D I
BAE86877.1 WP_017840377.1 WP_013818323.1 AAF04158.2 pdbl2MOBI AAC45291.1 WP_102844999.1	79K DRAGYWWLKATO DRAGYWWLKATO DRAGFWWLKATO DRAGYWWIKANO DKAGFWWIKADO DKAGFWWIKADO DRGGFWWIKADO	89A GKIEVDCEEVSELL GKIEVDCEEVSELL GKIEIDCVEVSELL GKIEVDCDEISELL GAIEIDAAEAGELL GAIEIDAAEAADLL GKIQIDTEKASDLL	99F GRTFSVYDFL GRTFSVYDFL GKHYSVYDLL GKPFSVYDLL GKPFSVYDLL GKTYSIYDFL	109S VDVS STIGRA VDVS STIGRA VDVS STIGRA INVS STVGRA VNVS STVGRA VNVS STIGRA	119G A FTL GE K FT I T A YTL GE K FT I T A YTL GE T FT I T A YTL G NK FT I T A YTL GTK FT I T A YTL G TK FT I T A YTL G NT FT I T	129M SELMGLDRKL SELMGLDRVK SELMGLDVKL SELMGLDRAL SELMGLDRAL SELMGLDRKL	+1 E DL K A A E DL K A A K DL A A A E D Y H A - T D I T D I T D A
BAE86877.1 WP_017840377.1 WP_013818323.1 AAF04158.2 pdbl2MOBI AAC45291.1 WP_102844999.1 VFU08594.1	79K DRAGYWWL KAT (DRAGYWWL KAT (DRAGFWWL KAT (DRAGFWWI KAN (DKAGFWWI KAD (DRGGFWWI KAD (DRGGFWWI KAD (89A GKIEVDCEEVSELL GKIEVDCDEVSELL GKIEVDCDEISELL GAIEIDAAEAGELL GAIEIDAAEAADLL GKIQIDTEKASDLL GKIQIDTEKASDLL	99F GRTFSVYDFL GRTFSVYDFL GKHYSVYDLL GRQFNVYDFL GKPFSVYDLL GKTSIYDFL GKTYSIYDFL	109S VDVS STIGRA VDVS STIGRA VDVS STIGRA INVS STIGRA VNVS STVGRA VNVS STIGRA VNVS STIGRA	I 19G A FTL GEKFT I T AYTL GEKFT I T AYTL GEKFT I T AYTL GNKFT I T AYTL GTKFT I T AYTL GNT FT I T AYTL GNT FT I T AYTL GNT FT I T	129M SELMGLDRKL SELMGLDRVL SELMGLDRKL SELMGLDRAL SELMGLDRAL SELMGLDRKL SELMGLDRKL	+1 E DL KAA E DL KAA KDL AAA E DYHA - T DI T DI T DA T DA T DV
BAE86877.1 WP_017840377.1 WP_013818323.1 AAF04158.2 pdbl2MOBI AAC45291.1 WP_102844999.1 VFU08594.1 WP_020174571.1	79K DRAGYWWLKATO DRAGYWWLKATO DRAGFWWLKATO DRAGFWWIKADO DKAGFWWIKADO DRGGFWWIKADO DRGGFWWIKADO DRAGFWWIKADO	89A GKIEVDCEEVSELL GKIEVDCEEVSELL GKIEVDCDEISELL GAIEIDAAEAGELL GAIEIDAAEAGLL GKIQIDTEKASDLL GKIQIDTEKASDLL GKIEVDTEKAADLL	99F GRTFSVYDFL GRTFSVYDFL GRQFNYDFL GKPFSVYDLL GKPFSVYDLL GKTYSIYDFL GKTYSIYDFL GKTYSIYDFL	109S VDVS S T I G A VDVS S T I G A VNVS S T VGR A VNVS S T I G A VNVS S T I G A VNVS S T I G A	1/19G A FTL GE K FT I T A YTL GE K FT I T A YTL GE K FT I T A YTL GTK FT I T A YTL GTK FT I T A YTL GTK FT I T A YTL GNT FT I T A YTL GNT FT I T	129M SELMGLDRKL SELMGLDRVKL SELMGLDRKL SELMGLDRAL SELMGLDRAL SELMGLDRKL SELMGLDRKL	+1 E DL KAA E DL KAA K DL AAA E D YHA - T D I T D A T D A T D V T D V T D I

Figure S1. Multiple sequence alignment of the regulatory MMOB protein in sMMO enzymes. The strictly conserved residues on the N-terminal MMOB tail that interact with Helices H and 4 of MMOH are highlighted in yellow. The strictly conserved residues in the core of MMOB that interact with MMOH Helices E and F are highlighted in cyan. Sequences compared include - BAE86877.1 - *Methylomicrobium japanense*; WP_017840377.1 *Methylomicrobium buryatense*; WP_013818323.1 *Methylomonas methanica*; AAF04158.2 *Methylococcus capsulatus* Bath; pdb|2MOB| *Methylosinus trichosporium* OB3b; AAC45291.1 *Methylocystis sp.* M; WP_102844999.1 *Methylocella silvestris*; VFU08594.1 *Methylocella tundra*; WP_020174571.1 *Methyloferula stellate*; KJB91174.1 *Skermanella aerolata* KACC 11604.

	-1	10A	20P	30K	40K	50Y	60F
WP 068635403.1	MS	ANMAVKOALK	ANPVPSSVDP	OEVHKWLODFTW	, DFKGKTAKYP1		EOFKLTAKE YAR
AAR98534.1	M S A	SNLAVKOSLK	AHP VPT SVDP	OEVHKWLODFTW	DFKAKSAKYP	KYDMDVNTRI	EOFKLTAKEYAR
WP 104955546.1	MLTASOS	GHKVLEOTKK	SVRVPTSVDP	OEVHRYLMDFKW	E F A E N R S K Y G	KYEMANMTKI	EOFKLTAKEYAR
WP 010960482.1	MALST	ATKAATDALA	ANRAPTSVNA	OEVHRWLOSFNW	DFKNNRTKYAT	T K Y K MANETKI	E Ô F KLIAKE <mark>Y</mark> AR
WP 153249048.1	MA I S A	ATKAATDALA	THRAPTSVNA	OEVHRWLOSFNW	E FKNNRTKYAT	T K Y K MANETKI	E Ô F KLIAKE <mark>Y</mark> A R
BAE86875.1	MA I S A	ATKAATDALK	INRAPVSVGA	QE VHRWL QSF SW	DFEKNRSKYPT	KYHMANDT KI	E Q F K L I A K E <mark>Y</mark> A R
WP 013818321.1	MA I S A	ATKAATDALA	ANP AP V S V G A	QEVHRWMQSFTW	DFDKNRTKYST	T K Y KMANDT KI	EQFKLIAKE YAR
WP 087143657.1	MA I S A	ATKAATDALA	VNRAPVSVGA	QEVHRWMQSFTW	DFEKNRTKYST	T K Y KMANDT KI	EQFKLIAKE YAR
BAJ17645.1	MA I S A	ATKAATDALA	VNRAP VSVGA	QEVHRWMQSFTW	DFEKNRTKYST	[KYKMANDTK]	EQFKLIAKE <mark>Y</mark> AR
CAA39068.2	MA I S L	ATKAATDALK	VNRAP VGVEP	QEVHKWLQSFNW	D F K E N R T K Y P T	[KYHMANE TK]	E Q F K V I A K E <mark>Y</mark> A R
PKO92487.1	MAISS	ATKAATDALS	ANRAPT SVGA	QEVHKWMQEFNW	D F K G N S T K Y P T	ſ K Y KMA N D T K I	E Q F K L I A K E <mark>Y</mark> A R
PCJ58204.1		M	IVA I P K V D V K P	QEVHKYLQEFGW	NMDKRRGKYP 1	T K Y T Y N P T A R I	E Q F K L I A K E <mark>Y</mark> C R
WP_046753692.1	M	TVIPGGF-RA	PTQREIKVGA	QDVHRWIQDMGW	EVPGGRSKYP1	T K Y E F D G G T R I	E Q F K L I V G E <mark>Y</mark> C R
WP_014211362.1	MT	STLPGKVTGR	P D A P H A S V N G	Q E V H S WL Q D L G W	DEATTRGKYP1	F K Y K F D P N A R I	E Q F K L V V R D <mark>Y</mark> G R
ACZ56334.1	MT	STLPGKVSGH	PSAPHASVSG	Q E V H S WL MD L G W	DSDTIRGKYP1	F K Y K F D P N A R I	E Q F K L V A R D <mark>Y</mark> G R
	70M	80G	90G	100G	110L	120A	130A
WD 069625402 1	MEGINEE	POVCTUDCI	DRIDACNEVII	D VWCE VMVI VCN	TELETCENCAL	CEALLWDTA.	DEBEORNOVI AO
WP_008053405.1	MESINEE	ROVETLIDEL	DRLDAGNKVH	P KWGE VMKL V SN DVWGE IMVI VAN	IFLEIGEIGAIA	AGSALLWDIA	Q S P E Q K NG I L AQ
WP 10/0555/6 1	MESIKEE	ROFGTLIDGI	DRIDAANRVH	P R WOE I MKL VAN	IFLEIGE IUAIA	AGSALLWDIA	Q S F E Q K NG T L AQ
WP_010060482.1	MEAVKDE	ROFGSLODAL	TRINAGVRVH	PKWNETMKVVSN	IFLEVGEVNAL	ATGMI WDSA	Q SI EQKNOTE SQ
WP 153249048 1	MESVKDE	ROFGSLODAL	TRLNAGHRVH	PKWNETMKVVSN	IFLEVGEYNAL	AT GML WD S A	TAAEOKNGYLAO
BAE86875 1	MESVKDE	ROFGSLODAL	TRLDAGNRIH	PKWGETMKVASN	FLEVGEYNAL	AT GML WD SA'	TAPEOKNGYLAO
WP 013818321.1	MESVKDE	ROFGSLODVL	TRVDAANRVH	PKWNESMKVISN	FLEVGEYNAL	AT GML WD SA'	TAPEOKNGYLGO
WP 087143657.1	ME SVKDE	ROFGSLODVL	TRVDAANRVH	P KWNE SMKVISN	FLEVGEYNAI	ATGMLWDSA	TAPEOKNGYLGO
BAJ17645.1	M <mark>E</mark> SV <mark>K</mark> DE	RQFGSLQDVL	TRVDAANRVH	P KWNE SMKVI SN	FLEVGEYNAI	AAT GML WD SA'	T A P E Q K N G Y L G Q
CAA39068.2	M <mark>E</mark> A A <mark>K</mark> D E	RQFGTLLDGL	TRLGAGNKVH	PRWGETMKVISN	FLEVGEYNAIA	A A S AML WD S A'	FAAEQKNGYLAQ
PKO92487.1	M <mark>E</mark> SV <mark>K</mark> DE	RQFGTLLDGL	TRLDAGNRVH	PRWGEAMKVVSN	FLESGEYNAIA	A A S AML WD S A'	T A A E Q K N G Y L A Q
PCJ58204.1	M <mark>E</mark> EE <mark>K</mark> DS	RQYGTLLDGL	TRMNVGSRIE	P R WNE I MKL G A P	LLEAGEYAAIS	SGTAVLWNAT	QSPELKNGYLAQ
WP_046753692.1	M <mark>E</mark> TE <mark>K</mark> DD	RQYGSLLDSL	ARLQAGGRIE	P R WAE AMK F V T T	FLELGEYSSIC	GGSAMLLDAV	VSPEQRNGYLAQ
WP_014211362.1	M <mark>E</mark> GE <mark>K</mark> DD	RQYGSLLDSL	ARLKAPTRVE	P R WAEMMKLLGC	GALELGEYNAL S	SGAAVMADTTI	RSPELRNGYLMQ
ACZ56334.1	M <mark>E</mark> GE <mark>K</mark> DD	RQYGSLLDSL	ARLKAPTRVE	PRWAEVMKLLAC	GALELGEYNAIA	AGSAVLADTTI	RSPELRNGYLMQ
		1500	160K	170D	180L 1	90D 2	200C 210A
WD 068635403 1	VIDEIPU	150Q	160K	170D	180L 1	90D 2	200C 210A
WP_068635403.1	V I <mark>D</mark> E I <mark>R H</mark> V I DE I PH	150Q VNQTAYVNYY VNOTAYVNYY	160K YGKHYYDPAG	170D HTNMRQLRAINP	180L 1 L Y P G V K R A F G I	90D 2 EGFLAGDAVE	200C 210A SSINLQLVGEAC
WP_068635403.1 AAR98534.1 WP_104955546.1	V I DE I RH V I DE I RH V I DE I RH	150Q VNQTAYVNYY VNQTAYVNYY TNOTAYVNYY	160K YGKHYYDPAG YGKHYYDPAG YSKHSYDAAG	170D HTNMRQLRAINP HTNMRQLRAINP	180L 1 LYPGVKRAFGI LYPGVKRSFGI	90D E G F L A G D A V E E G F L A G D A V E O G F N A G D P V E	200C 210A SSINLQLVGEAC SSINLQLVGEAC SSINLQLVGEAC
WP_068635403.1 AAR98534.1 WP_1049555546.1 WP_010060482.1	V I DE I RH V I DE I RH V I DE I RH V I DE I RH	150Q VNQTAYVNYY VNQTAYVNYY TNQTAYVNHY THOCAYVNYY	160K YGKHYYDPAG YGKHYYDPAG YSKHSYDAAG FAKNGODPAG	170D HT NMR QL R A I NP HT NMR QL R A I NP HNN AR QVR S VGP HND AR RT RT I GP	180L 1 L Y P G V K R A F G I L Y P G V K R S F G I A F R G I K R A F G I L W K G M K R V F S I	90D 2 E G F L A G D A VE E E G F L A G D A VE E D G F N A G D P VE E D G F I S G D A VE	200C 210A S S I NL QL V GE A C S S I NL QL V GE A C C S I NL QL V A E A C C S L NL QL V A E A C
WP_068635403.1 AAR98534.1 WP_104955546.1 WP_010960482.1 WP_153249048.1	V I DE I RH V I DE I RH V I DE I RH V I DE I RH V L DE I RH V L DE I RH	150Q VNQTAYVNYY VNQTAYVNYY TNQTAYVNYY THQCAYVNYY THQCAYVNYY	160K Y G K H Y Y D P A G Y G K H Y Y D P A G Y S K H S Y D A A G F A K N G Q D P A G F A K N G Q D P A G	170D HTNMRQLRAINP HTNMRQLRAINP HNNARQVRSVGP HNDARRTRTIGP HNDARRTRTIGP	180L 1 L Y P G V K R A F G I L Y P G V K R S F G I A F R G I K R A F G I L W K G M K R V F S I L W K G M K R V F S I	90D 2 G F L A G D A VE 3 G F L A G D A VE 3 O G F I A G D P VE 0 O G F I S G D A VE 0 O G F I S G D A VE 0	200C 210A SSINLQLVGEAC SSINLQLVGEAC CSINLQLVAEAC CSLNLQLVGEAC CSLNLQLVGEAC
WP_068635403.1 AAR98534.1 WP_1049555546.1 WP_010960482.1 WP_153249048.1 BAE86875.1	VIDEIRH VIDEIRH VIDEIRH VLDEIRH VLDEIRH VLDEIRH	150Q VNQTAYVNYY VNQTAYVNYY TNQTAYVNHY THQCAYVNYY THQCAYVNHY TNOCGYVNYY	l60K YGKHYYDPAG YGKHYYDPAG YSKHSYDAAG FAKNGQDPAG FAKNGQDPAG YTKHFHDPAG	170D HTNMRQLRAINP HTNMRQLRAINP HNNARQVRSVGP HNDARRTRTIGP HNDARRTRTIGP	180L 1 L Y P G V K R A F G I A F R G I K R A F G I L W K G M K R V F S I L W K G M K R V F S I L W K G M K R V F S I	90D 2 G G F L A G D A V E G G F L A G D A V E O G F N A G D P V E O G F I S G D A V E O G F I S G D A V E O G F I S G D A V E	200C 210A SSINLQLVGEAC SSINLQLVGEAC CSINLQLVAEAC CSINLQLVGEAC CSINLQLVGEAC CSINLQLVGEAC
WP_068635403.1 AAR98534.1 WP_104955546.1 WP_010960482.1 WP_0138249048.1 BAE86875.1 WP_013818321.1	VIDEIRH VIDEIRH VIDEIRH VLDEIRH VLDEIRH VLDEIRH VLDEIRH	150Q VNQTAYVNYY VNQTAYVNYY TNQTAYVNHY THQCAYVNHY TNQCGYVNYY TNQCAYINYY	I 60K Y G K H Y Y D P A G Y G K H Y Y D P A G Y S K H S Y D A A G F A K N G Q D P A G F A K N G Q D P A G Y T K H F H D P A G F A K O G O D A A G	170D HTNMRQLRAINP HTNMRQLRAINP HNNARQVRSVGP HNDARRTRTIGP HNDARRTRTIGP HNDARRTRAIGP	180L 1 L Y P G V K R A F G I L Y P G V K R S F G I L W K G K R A F G I L W K G M K R V F S I L W K G M K R V F S I L W K G M K R V F S I L W K G M K R V F S I	90D 2 E G F L A G D A V E E G G F L A G D A V E E O G F N A G D P V E E O G F I S G D A V E E O G F I S G D A V E E O G F I S G D A V E E	200C 210A S S I N L Q L V G E A C S S I N L Q L V G E A C C S I N L Q L V A E A C C S L N L Q L V G E A C C S I N L Q L V G E A C C S I N L Q L V G E A C C S I N L Q L V G E A C C S I N L Q L V G E A C C S I N L Q L V G E A C C S I N L Q L V G E A C
WP 068635403.1 AAR98534.1 WP 0104955546.1 WP 010960482.1 WP 153249048.1 BAE86875.1 WP 013818321.1 WP 087143657.1	V I DE I RH V I DE I RH V I DE I RH VL DE I RH	150Q VNQT A Y VN Y Y VNQT A Y VN Y Y TNQT A Y VN H Y THQC A Y VN H Y TNQC G Y VN Y Y TNQC G Y IN Y Y TNQC G Y IN Y Y	IGOK YGKHYYDPAG YGKHYYDPAG YSKHSYDAAG FAKNGQDPAG FAKNGQDPAG YTKHFHDPAG FAKQGQDAAG FAKQGQDAAG	170D HTNMRQLRAINP HNNARQVRSVGP HNDARRTRTIGP HNDARRTRTIGP HNDARRTRTIGP HNDARRTRAIGP HNDARRTRAIGP	180L I L YP GVKRAFGI A FRG I KRAFGI L WKGMKRVFSI L WKGMKRVFSI L WKGMKRVFSI L WKGMKRVFSI L WKGMKRVFSI	90D 2 E G F L A G D A V E E G F L A G D A V E O G F N A G D P V E O G F I S G D A V E O G F I S G D A V E O G F I S G D A V E O G F I S G D A V E O G F I S G D A V E	200C 210A SSINLQLVGEAC SSINLQLVGEAC CSINLQLVGEAC CSLNLQLVGEAC CSINLQLVGEAC CSINLQLVGEAC CSINLQLVGEAC CSINLQLVGEAC
WP 068635403.1 AAR98534.1 WP 104955546.1 WP 010960482.1 WP 153249048.1 BAE86875.1 WP 013818321.1 WP 087143657.1 BAJ17645.1	V I DE I RH V I DE I RH V I DE I RH VL DE I RH VL DE I RH VL DE I RH VL DE I RH VMDE I RH VMDE I RH	1,50Q VNQT A Y VNY Y VNQT A Y VNY Y TNQT A Y VNH Y THQC A Y VNH Y TNQC G Y VNY Y TNQC G Y VNY Y TNQC G Y I NY Y TNQC G Y I NY Y	I 60K Y G K H Y Y D P A G Y G K H Y Y D P A G Y S K H S Y D A A G F A K N G Q D P A G F A K N G Q D P A G F A K Q G Q D A A G F A K Q G Q D A A G F A K Q G Q D A A G	170D HTNMR QL R A I N P HTNMR QL R A I N P HNN A R Q V R S V G P HND A R R T R T I G P HND A R T R T I T I G P HND A R T R A I G P HND A R R T R A I G P HND A R R T R A I G P	180L 1 L YP GVKR AF GI AFRG I KR AF GI L WKGMKR VF SI L WKGMKR VF SI L WKGMKR VF SI L WKGMKR VF SI L WKGMKR VF AI L WKGMKR VF AI	90D 22 E G F L AGDA V E D G F L AGDA V E O G F I S GDA V E	200C 210A S S I N L Q L V GE A C S S I N L Q L V GE A C C S I N L Q L V GE A C C S L N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C
WP 068635403.1 AAR98534.1 WP 104955546.1 WP 010960482.1 WP 013960482.1 BAE86875.1 WP 013818321.1 WP 013818321.1 BAJ17645.1 CAA39068.2	VIDEIRH VIDEIRH VIDEIRH VLDEIRH VLDEIRH VLDEIRH VLDEIRH VLDEIRH VLDEVRH VLDEIRH	150Q VNQTAYVNYY TNQTAYVNYY TNQTAYVNHY THQCAYVNYY TNQCAYVNYY TNQCGYINYY TNQCGYINYY TNQCGYINYY TNQCGYINYY	I,60K YGKHYYDPAG YGKHYYDPAG YSKHSYDAAG FAKNGQDPAG FAKNGQDPAG FAKQGQDAAG FAKQGQDAAG FAKQGQDAAG YSKHYHDPAG	170D HTNMRQLRAINP HNNARQVRAINP HNDARRTRTIGP HNDARRTRTIGP HNDARRTRAIGP HNDARRTRAIGP HNDARRTRAIGP HNDARRTRAIGP	180L I L YP GVKRAFGI AFRGIKRAFGI UWKGMKRVFSI LWKGMKRVFSI UWKGMKRVFSI LWKGMKRVFAI LWKGMKRVFAI LWKGMKRVFAI LWKGMKRVFAI	90D G G F L A G D A V E G G F L A G D A V E O G F I S G D A V E O G F I S G D A V E O G F I S G D A V E O G F I S G D A V E O G F I S G D A V E O G F I S G D A V E	200C 210A S S I N L Q L V GE A C S S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C
WP 068635403.1 AAR98534.1 WP 010960482.1 WP 010960482.1 WP 0133249048.1 BAE86875.1 WP 013818321.1 WP 087143657.1 BAJ17645.1 CAA39068.2 PKO92487.1	V I DE I RH V I DE I RH V I DE I RH VL DE I RH VMDE I RH VL DE V RH VL DE V RH VL DE I RH	150Q VNQTAYVNYY TNQTAYVNYY THQCAYVNYY THQCAYVNYY THQCGYVNYY TNQCGYINYY TNQCGYINYY TNQCGYINYY TNQCGYINYY TNQCGFINYY	IGOK YGKHYYDPAG YGKHYYDPAG YSKHSYDAAG FAKNGQDPAG FAKNGQDPAG FAKQGQDAAG FAKQGQDAAG FAKQGQDAAG FAKQGQDAAG YSKHYHDPAG	170D HTNMR QL RAINP HNNAR QVR SVGP HNDARRTRTIGP HNDARRTRTIGP HNDARRTRAIGP HNDARRTRAIGP HNDARRTRAIGP HNDARRTRAIGP HNDARRTRAIGP HNDARRTRAIGP	180L I L YP GVKRAFGI AFRGIKRAFGI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFAI UWKGMKRVFAI UWKGMKRVFAI	90D 22 CGFLAGDAVE CGFLAGDAVE CGFLSGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE	200C 210A S S I N L QL VGE A C S S I N L QL VGE A C S S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S I N L QL VGE A C C S V N L QL VGE A C C S V N L QL VGE A C C S V N L QL VGE A C
WP 068635403.1 AAR98534.1 WP 0104955546.1 WP 010960482.1 WP 013249048.1 BAE86875.1 WP 081343637.1 BAJ17645.1 CAA39068.2 PK092487.1 PCJ58204.1	VIDEIRH VIDEIRH VIDEIRH VLDEIRH VLDEIRH VLDEIRH VLDEIRH VLDEIRH VLDEVRH VLDEIRH VLDEIRH TMDEVRH	150Q VNQTAYVNYY TNQTAYVNYY THQCAYVNYY THQCAYVNYY TNQCGYVNYY TNQCGYVNYY TNQCGYINYY TNQCGYINYY TNQCGYINYY TNQCGYINYY TNQCGYINYY	IGOK YGKHYYDPAG YGKHYYDPAG YSKHSYDAAG FAKNGQDPAG FAKNGQDPAG FAKQGQDAAG FAKQGQDAAG FAKQGQDAAG FAKQGQDAAG FAKQGQDAAG FSKHYHDPAG FSKHYHDPAG MAKHTYDPSG	170D HTNMR QL R A I N P HN NA R Q V R S V G P HN D A R T R T I G P HN D A R T R T I G P HN D A R T R A I G P HN D A R R T R A I G P HN D A R T R A I G P HN D A R T R A I G P HN D A R T R T I G P HN D A R T R T I G P HN D A R T R T I G P HN D A R T R T R M N P	180L 1 L YP GVKRAFGI L YP GVKRAFGI AFRGIKRAFGI UKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFSI UWKGMKRVFAI UWKGMKRVFAI UWKGMKRVFAI UWRPAKRALSI	90D 22 EGFLAGDAVE EGFLAGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE OGFISGDAVE	200C 210A S S I N L Q L V GE A C S S I N L Q L V GE A C S S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S I N L Q L V GE A C C S V N L Q L V GE A C C S V N L Q L V GE A C C S V N L Q L V GE A C C S I N L Q L V GE A C C S V N L Q L V GE A C
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Figure S2. Multiple sequence alignment of a portion of the α-subunit of sMMOH in sMMO enzymes. The strictly conserved residues of the hydrogen bonding network distal to the histidine ligands are highlighted in yellow. The strictly conserved Pro215 residue in Helix E is highlighted in cyan. Sequences compared include the α-subunit of hydroxylase proteins for a butane monooxygenase - AAR98534.1 *Brachymonas petroleovorans*. The remaining sequences are for the α-subunit of hydroxylase proteins for methane monooxygenases - WP_068635403.1 *Thauera butanivorans*; WP_104955546.1 *Sphingobium sp.* SCG-1; WP_010960482.1 *Methylococcus capsulatus* Bath; WP_153249048.1 *Candidatus Methylospira mobilis*; BAE86875.1 *Methylomicrobium japanense*; WP_013818321.1 *Methylomonas methanica*; WP_087143657.1 *Crenothrix polyspora*; BAJ17645.1 *Methylovulum miyakonense* HT12; PKO92487.1 *Betaproteobacteria bacterium* HGW-Betaproteobacteria-1; PCJ58204.1 *Rhodospirillaceae bacterium*; WP_046753692.1 *Mycolicibacterium elephantis*; WP_014211362.1 *Mycolicibacterium rhodesiae*; ACZ56334.1 *Mycolicibacterium chubuense* NBB4.



Figure S3. The B-factors of the MMOB protein in the diferric sMMOH:MMOB structure are represented as a putty cartoon figure in magenta. The MMOH surface is represented in green. Thicker secondary structure elements indicate high B-factor while thin regions indicate low B-factors.



Figure S4. Surface representation of the sMMOH active site cavity depicts the difference in polarity in diferric MMOH (panel A) and the diferric MMOH:MMOB complex (panel B) as a result of the reorganization of Thr213.The side-chain of residues that project into the active site cavity are colored according to the following scheme; carbon atoms = cyan in MMOH, green in MMOH:MMOB; oxygen atoms = red; nitrogen atoms = blue. The iron atoms are shown as orange spheres.



Figure S5. The diiron clusters of the diferric sMMOH:MMOB complex (6YD0, panel A) and diferrous sMMOH:MMOB complex (6YDI, panel B) are colored according to the atomic B-factors. A color spectrum from red to white to blue represent the B-factors, where red = 45, white = 35 and blue = 25.



Figure S6. Diferrous sMMOH:MMOB crystals washed of reductant undergo reoxidation upon exposure to O_2 in the reaction region of the DOT (drop on tape) system while staying reduced if exposed to helium under identical conditions. Panel A: 2Fo-Fc map of the diiron cluster of sMMOH^{red}:MMOB immediately prior to O_2 exposure (6YY3, top) and after post-4s O_2 exposure, 6 s aging (6YDU, bottom) contoured to 2 σ . Panel B: An alignment of the diiron cluster of 6YY3 (grey atoms) with that of the diferrous sMMOH:MMOB complex (6YDI; yellow atoms) indicates that the sMMOH:MMOB crystals stay reduced in the absence of O_2 after removal of the dithionite reductant. Panel C: An alignment of 6YDU (purple atoms) with that of the diferric sMMOH:MMOB complex (6YD0; green atoms) indicates that the reduced sMMOH:MMOB crystals oxidize completely to the resting diferric state in this timeframe.



Figure S7. Loss of unassigned electron density (Fo-Fc electron density map (green mesh) contoured at 3σ , 2Fo-Fc electron density map (blue mesh) contoured at 2σ) from the active site post *in-crystallo* single turnover. Panel A: Diferric MMOH:MMOB complex (6YD0), Panel B: Reduced MMOH:MMOB complex (6YDI) and Panel C: Re-oxidized MMOH:MMOB complex (6YDU).



Figure S8. Top-down perspective of the hydrogen-bonding network on the distal side of the diiron cluster. The residues are colored according to its B-factor where blue indicates the lowest B-factor (25), red the highest (45) and white an intermediate value (35). The iron atoms are represented as larger spheres while the bridging μ -hydroxo groups are shown as smaller spheres. The active site cavity is shown as a grey surface. Black dashed lines depict the trans-positioning of the histidine ligands with respect to the μ -hydroxo moiety facing the active site. Red and blue dashed lines show the hydrogen bond network.

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