Supplemental Data

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Supplemental Data, **Figure S1** (continued on next two pages)

Alignment of the *A. marina* full length catalase-related hemoprotein/lipoxygenase sequence (GenBank accession number ABW27596) with sequences from *Plexaura homomalla* (AF003692) *Ostreococcus lucimarinus* (XM_001418864) and *Anabaena* PCC 7120 (NP_478445.1). The alignment was performed using the Clustal W program in DNAStar with some manual adjustments (mainly to remove spaces corresponding to the different lengths of the N-termini and to make one single long space for the shorter LOX domains of *A. marina* and *Anabaena*).

Page 1: Highlighted in **Red** are the distal heme His and Asn (Ser in *A. marina*) and the Tyr proximal heme ligand. In **Blue** are the Thr next to the distal His and the consensus sequence around the proximal Tyr ligand, RxxxYxxxxxR)

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14 41 21 16	K G E	K K L Q	K I V I K J	. I КК И Q : М	S I E I L I	N I K F K I E L	A K A Q A	R A T L = M A M	F (F ⁻ F ^y	GR TL VF SA	G G A	A Q T T T L G L	I L A	K I Q L	G – G F K – R –	R R G	R A R A R F	A T A T 5 T		SY TV PV TD	G G G	TV TG VG II	G G V	K G I G I G K G	V E Q N	L I F	KV TIV TVI TVI	V I	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
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273 292 289 264	P V L V A V I V	E D G D	E E K E T E P 1	D T - N T	H H Y	PF W F W	H [F [L]		A A G A	V L V V A I	D S R V	L G I K L H L N	S T I Q	I P A D	L P L S L P E S	D P Q E	- E D \ H S N [A - - Y	E A E K E N Q E	T I I	E F A F S F A Y	N G N	PY IA MN G	N N R	A Q T	РР РА 9 РА 0 Н Ү 1	E S C D	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
312 331 325 304	L S L C M F		II LE TF	A A A A A A A A A A A A A A A A A A A	K K I Y	TA P NS V	R E Q I D I	T Y Y Y F	A N N A	5 V 5 I 5 L 5 L	N G N G	HL EL WT VS	R R G	S V A A	V V A V V V L V	Y Y H	Q 1 T V E 1 Y F	IS VV S G	A Q Y S	N M H L K I I V	R R R R	K Y K L R V A E	′Q /F R	TP TR TQ	S K G Y	S S I (R H L Y	SL GSL CD YG 2	V	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX

Alignment **Figure S1**, second page This shows the first half of the LOX domains Highlighted in **Red** are the first two ligands to the lipoxygenase non-heme iron

352 368 365 344	- V Y -	- P A -	- A P -	- G E -	Q A -	- N S -	- K -	- I K -	- Y -	- N T -	V I -	E K -	- V -	- E T -	- T T -	- G H -	– D S –	R I -	E A -	- H G -	- A S -	- G -	- T I -	- D D -	A G	- T D -	I V -	T V -	I L -	- R Q -	- I F -	T V	G G	 A T 	K (R A	T D T P	 	- Y K	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
352 408 405 344	L I -	- K V -	L	– D H –	– K D –	 W 	E F L	– H G	– N D –	- D S -	F F	E A	_ А Т -	G G	- S A -	- K I -	– E S	Q T -	- Y H -	– T F	V V	Q H	G A -	F D -	– D D	- V I -	G G	D E	- I P -	Q A -	L Y	I A -	- E I	L I !	H 9 5 (5 [2 -	-	G G 	(- G -	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
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352 487 478 344	- P L -	- G P -	- E C -	- A S -	- T I -	- L T	- P -	F T	- N T -	- E S -	- V L -	- P A -	- - C -	- R -	A Q -	- I L -	V I -	D S A	W E D	G Q A	S R T -	G Q E -	H K Q -	Q E Y	Р Ц –	S E S	L Q F	P R M	E K F	Q L K -	Y T E -	P Y F	Q Q	G 1 W [W 9	Г () `` 5 	G H Y V V (< 5	T P S D S Y) <u>-</u>	5	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
375 525 517 344	F M L	D P K	N G D	T N H D	K I S L	Р К Т Р	L A Y G	P D K	A T D P	R H V	V D S Y	K D F	P L P	K P L	P R I P	R D T V	Y V E T	Q Q E	A F I	N T S P	F D S S	G E T K	K K R	K S F	R K	I S D F	P Y F L	N Q F L	Q E V	Q S D K	L R T Y	F N	- 4 / V F	D F A I G I L T		D I V N E N	V V	PE LC KC SY	l V V	I V P	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
413 565 557 383	G G A S	I S D	– L M G	F I E	T T K	G M D	A F H	L E D	D N E K	- W I	L D K E	M S A	G Y L	T D D V	S D D S	V Y A	V H Y	S I R P	Y L T	M Y T	P R A	P N S L	N W D	I - L	T L A	R G P V	_ P G	- V T	– P A –	- N I -	M I T	R R D	L D P	DI RV -V	K F V F D T	= 4 H E R 9 E F	5	D D D R D E D S	F F F	F N E Y	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
446 605 593 419	F F F	V G L	Y R	R Q Q R	R F R	L M	N N N N	G G G	F A T Y	N H N	P P P	G V N G	K I Q A	L V I	N T R	R R E	V C S	T D S S	G A Q G	H L I Q	P P E	A S G	W - W	Q N K T	Y F H	Q P R E	V V L	C T T	Y N E H	D E N	C H L	S V A	K N R	H I G Y Y I	Q S V	V E F I H		P A R G P G		G K L	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
485 644 632 459	I N T H	L L F	P D E P	T E S D	K E	I I L	Т К А	- D A -	G	- H H	- I I	Y F	- I M -	V V	- D D	- F Y	- E -	- V I -	- L L	- - -	- G -	- A -	- К -	- S -	- Y -	- G -	- G -	- P -	v -	L _ _	- E -	- D (-	- [(-	 G ` 	Y	< \		 - E	F C	- - -	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
492 684 653 463	- L I -	- K -	– H R	- D G -	- E Q -	A A G	R D -	F - F	N R - V	F Y Q	C C C C	G A V R	Q A E L	- N -	- P S -	- L M -	- A -	a L L	F F F	Y F F	V A V	H N D	PKKK	H L S Q	S G T N	- H G	I L V	Q M R K	F P L	- I H	T A C S	L I I	Q Q K	G (I - I [2 1 1 1 1 1	r Q F (2 ! 	T Q P G S G E I	F A T	р А Г	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
519 723 689 490	P E S P	G N A C	– P –	I V	W W	T T	- P -	- Н К	E D	- E G -	- N -	D E Q	H Y E	N D Q	W W W	E M L Q	W M L Y	S A A A	к к к к	R F A R	U W H T	F L Y	R G M L	C V Q	A S A	E D E	F G F	V N L	F V S	Q H H Q	Q Q E	A M L	Q N K	S S L		(G	R T G T R C			A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX

Alignment **Figure S1**, third page This shows the second half of the LOX domains Highlighted in **Red** are the third and fourth iron ligands to the lipoxygenase domains and in **Blue** is the Gly-Ala determinant of R-S LOX specificity (Coffa and Brash, PNAS 101:15579, 2004)

550 763 728 521	MNLD LTTE LLME FNIE	QYA SFA PWS QYV	MAY LST VCV MAI	YR WR ER KR	NVV- NLAS TLPR RLAP	N N P A H P N H P T H P	IRLL VFKL VYRV VRAF	LEP LQP LRP INP	H L E G H I Y G H L I Y H L E G	LLSI VLAI VIAI LIFI	NKLGAN DTIGRK NTLGRS NSSAVP	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
589 803 768 561	LISG ELIG LLIS KIIG	PTG SGG PGG STG	FIP IVD VTD FIP	EA QS RV IA	SSLT SLC VAVG SMLT	PES GGGG QGG QGS	VDDV HVTF HMDL IVDV	LKD MEK MSK MKN	EISH CFKE AYQR ELSK	LSYH VNLQ FKLD LSYM	W T P H R Q D Y H L P N D L H V P T W N P I A D	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
629 843 808 601	TL ALKK SFKK -L	(RGV (RGV	P D D P L S E P	DR SK GD RD	VLNN LPGF LRGY IPGD	HYD YYR HHR LFT	PAAI DDGL DDSL PAAT	A M W A L W R A W A Y W	N L L T E A I E A C L R E L L N	QYVR TFIG RFAK NYVE	E F F E D H E I I A I F S I F K L H Q G L L	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
662 883 848 631	Q A G M Y K N D Y T S D Q P F E	1 E E Y 1 D D V 1 A E V D E L	WSE KRD AAD RTE	N E P Y	IQAM IQSW IQSM VNAI	SHD IYD ILE QVD	LVTH VHKN MQGY ELFA	SIL GWR GYQ ELK	- K P E V N P G - G T D - E R S	L – – G H Q D H R S Q H L Y S G	T L A V Q - G V P A S F G V P G S I D Q P P K Y	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
696 923 887 668	NNAD ESRE DSID DSSE) - L Q Q L K Q L V - L K	QLC EVL DIC SLL	VY TS TS MY	VIFL LVFT VMYT IIYH	S S F F S C C S F S S F	FHSW QHAA THAA LHSW	V N N V N F V N F A N F	K Q Y E S Q K D S Q W D K Q Y D	DGGD HYGF YYSY DAGN	V S Y S T I T P N A P A C P N R P L P N H V S M	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
735 963 927 707	GLWD VLRH IMRK G	0 T R H I P P P C P A P - D Y	PK- KKK TKK SQ-	G E J V P	ATLQ VTEK	Y S I L D V I Y	DPLR STLP DALP DQQT	V A E S K S S V G Q D K	REAK QAAK QAAQ IRFS	Q V T L A I A T T M A T Q R S L	L W T L S H V Y I L T K G W T L S Q T W V L S S	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
766 1003 967 734	V F S E D F S K E I	ERY EVF	R LGN VGH R	YN YS YI YN	PIMD ATAW TDMM SVAV	VGP EDK ITP YGS	TALK DALD AEIA DLLK	N L - A I N A L Q Q L -	R F Q D D L R S 	KLED ELRA	ISKKIK MGRQIE	A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX
783 1043 1007 751	LW QRNE NRNA IR	Q Q R N L E K L G E K S	QHI V-P VKA SIL	E P Y I Y P E P	GIPL YLLP YMHP GLPL	ANL ERI ERV	MMST PNGT PSNI MMSI	N I A I G V N I .				A Marina catLOX P Homomalla catLOX Ostreococcus catLOX Anabaena catLOX



Scheme 1. Analysis of the epoxide stereoconfiguration in the linoleic acid-derived epoxyalcohol product of the *A. marina* enzyme by chemical transformations.

Figure S2. SDS-PAGE and UV-Vis spectrum of *A. marina* fusion protein purified by Ni-NTA affinity column. Panel A: SDS-PAGE indicating an expected 92 kD fusion protein. Panel B: the UV-Vis spectrum showing the main Soret band at 405 nm and the 10 fold expanded y-axis showing more Soret bands at the longer wavelength 500, 535 and 619

 أ ОН

H₃COOC

nm. The absorbance ratio for $A_{405/280}$ is 0.52, demonstrating a good incorporation of heme in the purified protein.



Identification of peak 1 by GC-MS



Peak 1 (refers to main text Figure 2A) compound was converted to methyl ester TMS ether derivative and hydrogenated. GC-MS spectrum showed a base ion at m/z of 131 (100%), which is the α -cleavage between C15 and C16. The ion of m/z 144 (93%) was a rearrangement ion produced by cleavage between C14 and C15, followed by H migration from C15 to the carbonyl oxygen (H.W. Gardner et al, Lipids, Vol. 10, P602-608). Other ions are at m/z of 73 (75%), 159 (11%), 187 (5%), 281 (11%, 371-90[TMSOH]), 371 (7%, M-29), 385 (9%, M-15). The fragmentation pattern confirmed a structure of 13-keto-16-hydroxy-octadeca-9,14-dienoic acid for peak 1. Alternatively, peak 1 compound was converted to TMS ester TMS ether methoxime derivatives (with and without

hydrogenation). GC-MS spectrum again confirmed the structure as a γ -ketol of 13-keto-16-hydroxy-octadeca-9,14-dienoic acid.

O H₃CO	9	10 12 13 14 10 15 16 HO O	`
	Chemical shift	Multiplicity	Assignment, coupling constant
	5.57	m	H10
	5.45	m	H9, H15, H16
	3.99	dt	H12; $J_{12,11} = 10.9$ Hz, $J_{12,OH} = 5.2$ Hz
	3.39	d	-OH (on C12); $J_{12,OH} = 5.2$ Hz
	3.36	S	-OCH ₃
	2.97	dd	H11a; $J_{11a,12} = 6.8$ Hz, $J_{11a,10} = 17.8$ Hz
	2.80	dd	H11b; $J_{11b,12} = 7.0$ Hz, $J_{11b,10} = 17.8$ Hz
	2.45	m	H14a
	2.23	m	H14b
	2.11	t	H2, $J_{2,3} = 7.4$ Hz
	1.95	m	H8
	1.83	quintet	H17; J _{17,16} = 14.9Hz, J _{17,18} = 7.5Hz
	1.55	m	H3
	1.25	m	Н7
	1.17	m	H4, H5, H6
	0.84	t	H18; J _{18,17} = 7.6Hz

Table 1. ¹H-NMR chemical shift of α -ketol (12-hydroxy-13-keto-octadeca-9,15-dienoic acid). NMR spectra were acquired at room temperature in d-benzene solvent.

Table 2. ¹H-NMR chemical shift of the cyclopentenone. NMR spectra were acquired at room temperature in d-benzene solvent.

$O_{14} 12 10 9 \\ 15 16 17 18 10 9$	COOCH3	
Chemical shift	Multiplicity	Assignment, coupling constant
7.01	dd	H15; J _{15,14} = 5.8Hz, J _{15,16} = 2.7Hz
5.99	d	H14; $J_{14,15} = 5.9$ Hz
5.45	quintet	H9, H10; $J_{9,10} = 10.6$ Hz
3.36	S	-OCH ₃
2.72	m	H11a
2.33	m	H16

2.19	m	H11b
2.12	t,m	H2, H12
2.02	m	H8
1.55	quintet	H3
1.49	m	H17a
1.29	m	H7
1.18	m	H4, H5, H6
0.83	m	H17b
0.65	t	H18; $J_{18,17} = 9.1$ Hz

Figure S3. SP-HPLC analysis of the side chain transformation in alkali treated cyclopentenone molecule. The analysis was performed with the solvent hexane/isopropanol (100/1.5, v/v) using the silica column (4.6x250mm) at the flow rate of 1 ml/min.



The alkali treatment to the cyclopentenone showed a peak shift to the left on SP-HPLC, confirmed a cis-trans transformation due to the alkali treatment, indicating a cis arrangement of the two side chains in cyclopentenone molecule.

Products analysis of C18.403 incubation with A. marina fusion protein by GC-MS

1. γ-ketol identification:





GC-MS spectrum of peak1(refers to main text Figure 2B) TMS ester TMS ether derivative showed ions of m/z at 73 (100%), 131 (17%), 321 (18%), 423 (0.2%, M-29), 437 (0.3%, M-15), and 452 (0.1%, M). These ions confirmed a molecular structure of 16-hydroxy-13-ketol-octadec-6,9*Z*,14*E* trienoic acid, γ -ketol.



GC-MS spectrum of peak 2 (refers to main text Figure 2B) TMS ester TMS ether derivative showed ions of m/z at 73 (100%), 355 (60%), 97 (15%), 437 (3%, M-15), and 452 (0.2%, M), indicating a molecular structure of 12-hydroxy-13-ketol-octadec-6,9Z,15Z trienoic acid, α -ketol.

3. Cyclopentenone identification:





GC-MS spectrum of peak 3 (refers to main text Figure 2B) TMS ester TMS ether derivative showed ions of m/z at 347 (100%, M-15), 362 (61%, M), 73 (60%), 333 (20%, M-29). These ions confirmed a molecular structure of cyclopentenone.

Figure S4. RP-HPLC profile of γ -ketol, α -ketol and cyclopentenone from incubation of C20.4 ω 6 with *A. marina*. The RP-HPLC was carried out using a Waters Symmetry C18 column (25 x 0.46 cm), a solvent system of methanol/water/acetic acid in the proportions 70/30/0.01 (v/v/v), a flow rate of 1 ml/min and with UV detection at 205 and 235nm.



Peaks 1, 2, 3 of the RP-HPLC profile correspond to γ -ketol, α -ketol and cyclopentenone, the hydrolysis and cyclization products from the allene oxide. They were identified by GC-MS (data not shown) and the typical UV spectra.

Table 3. ¹H-NMR chemical shift of α -ketol (9-hydroxy-10-keto-octadec-12Z-enoic acid). NMR spectra were acquired at room temperature in d-benzene solvent.

O H ₃ CO	9 10 HO O	
Chemical shift	Multiplicity	Assignment, coupling constant
5.59	m	H12
5.48	m	H13
3.95	m	Н9
3.38	S	-OH
3.35	S	-OCH ₃
2.98	dd	H11a; $J_{11a,12} = 14.3$ Hz, $J_{11a,13} = 6.0$ Hz

2.81	dd	H11b; $J_{11b,12} = 14.3$ Hz, $J_{11b,13} = 7.1$ Hz
2.09	t	H2; $J_{2,3} = 7.1$ Hz
1.87	dt	H14; $J_{14,13} = 12.4$ Hz, $J_{14,15} = 6.2$ Hz
1.58	m	H8b
1.53	m	H3
1.32-1.18	m	H4, H5, H6, H7, H8a, H15, H16, H17
0.87	t	H18; $J_{18,17} = 7.1$ Hz

Figure S5. 13-hydroxy stereoconfiguration analysis by Chiralpak AD column. The analysis was performed with the solvent hexane/ethanol (100/5, v/v) using the Chiralpak AD column (4.6x250mm) at the flow rate of 1 ml/min.



The three panels on the left side are from enzymatic product alone, 13S-HODE derived epoxyalcohol and coinjection of enzymatic product with the 13S-HODE derived epoxyalcohol, respectively. The13S-HODE derived epoxyalcohol cochromatographed with the enzymatic product at the retention time 14.2 min, indicating a 13S-hydroxyl stereoconfiguration in the enzymatic epoxyalcohol. The three panels on the right side are from enzymatic product alone, 13RS-HODE derived epoxyalcohol and coinjection of enzymatic product with the 13RS-HODE derived epoxyalcohol and coinjection of enzymatic product with the 13RS-HODE derived epoxyalcohol, respectively. The results further confirmed a 13S-hydroxyl configuration in the enzymatic product.