SUPPLEMENTAL DATA

SUPPLEMENTAL TABLE 1. Overview of the stereochemistry of the detected products in the reaction of CspLOX2 with different fatty acids. Product hydroxides were analyzed using a a Chiralcel OD-H column (150 × 2.1 mm, 5 µm particle size, Daicel, VWR) with a solvent system of *n*-hexane/2-propanol/trifluoroacetic acid (100:5:0.1, v/v/v) at a flow rate of 0.1 ml/min. n.a.: not analyzed.

Substrate	Products	*R*/*S* ratio
18:3(n-3)	11-HOTE	n.a.
	13-HOTE	20/80
	12-HOTE	n.a.
	16-HOTE	65/35
	9-HOTE	83/17
	9,16-diHOTE 1	n.a.
	9,16-diHOTE 2	n.a.
20:4(n-6)	13-HETE	n.a.
	12-HETE	35/65
	15-HETE	14/86
	11-HETE	92/8

SUPPLEMENTAL FIGURE 1**. Partial amino acid sequence alignment of *Cyanothece* sp. CspLOX2 (accession number YP_002373243), *Nostoc punctiforme* NpLOX2(accession number ZP 00106490), *Plexaura homomalla* Ph8*R*-LOX(accession number AAC47743) and *Gaeumannomyces graminis* Mn-LOX (accession number AY040824).** The alignment was done using T-Coffee. The five amino acid iron ligands and the described determinants of regio- and stereospecificity have been marked with an asterisk and li, bo (according to (1)), sl (according to (2)) and co (according to (3)), respectively.

SUPPLEMENTAL FIGURE 2. **Relative product amounts formed by CspLOX2 from 18:2(n-6), 18:3(n-3) and 20:4(n-6).** CspLOX2 (15 µg) was incubated with 250 µg of the respective substrate in 200 mM sodium borate buffer pH 9.5 and the products were reduced by $SnCl_2$. Analysis was carried out by RP-HPLC/MS^2 and SP-HPLC. The figure shows mean values and standard deviations, which were calculated from three different experiments.

SUPPLEMENTAL FIGURE 3. **Reaction of CspLOX2 with 9*R*-HPOTE.** A) Overlay of UV spectra of the reaction of CspLOX2 (10 µg) with 9*R*-HPOTE (40 µM). The sample was scanned from 220 to 340 nm before addition of the substrate (t=0 min) and the reaction was completed after 9 min. One arrow indicates the decrease in absorbance at 235 nm and another indicates the increase at 269 nm, which was observed during the reaction. B) RP-HPLC-MS^2 analysis of the reaction products of CspLOX2 with 9*R*-HPOTE (*m/z* 309 → full scan). Peaks 1 and 2 contained 9,16-diHOTE. C) RP-HPLC-MS^2 analysis of the reaction products of CspLOX2 with 9*R*-HPOTE (*m/z* 291 → full scan). Peak 3 contained 9-KOTE.

References

1. Borngräber, S., Kuban, R. J., Anton, M., and Kühn, H. (1996) *J. Mol. Biol.* 264, 1145-1153
2. Sloane, D. L., Leung, R., Craik, C. S., and Sigal, E. (1991) *Nature* 354, 149-152
3. Coffa, G., and Brash, A. R. (2004) *Proc. Natl. Acad. Sci. USA* 101, 15579-15584

Figure 1

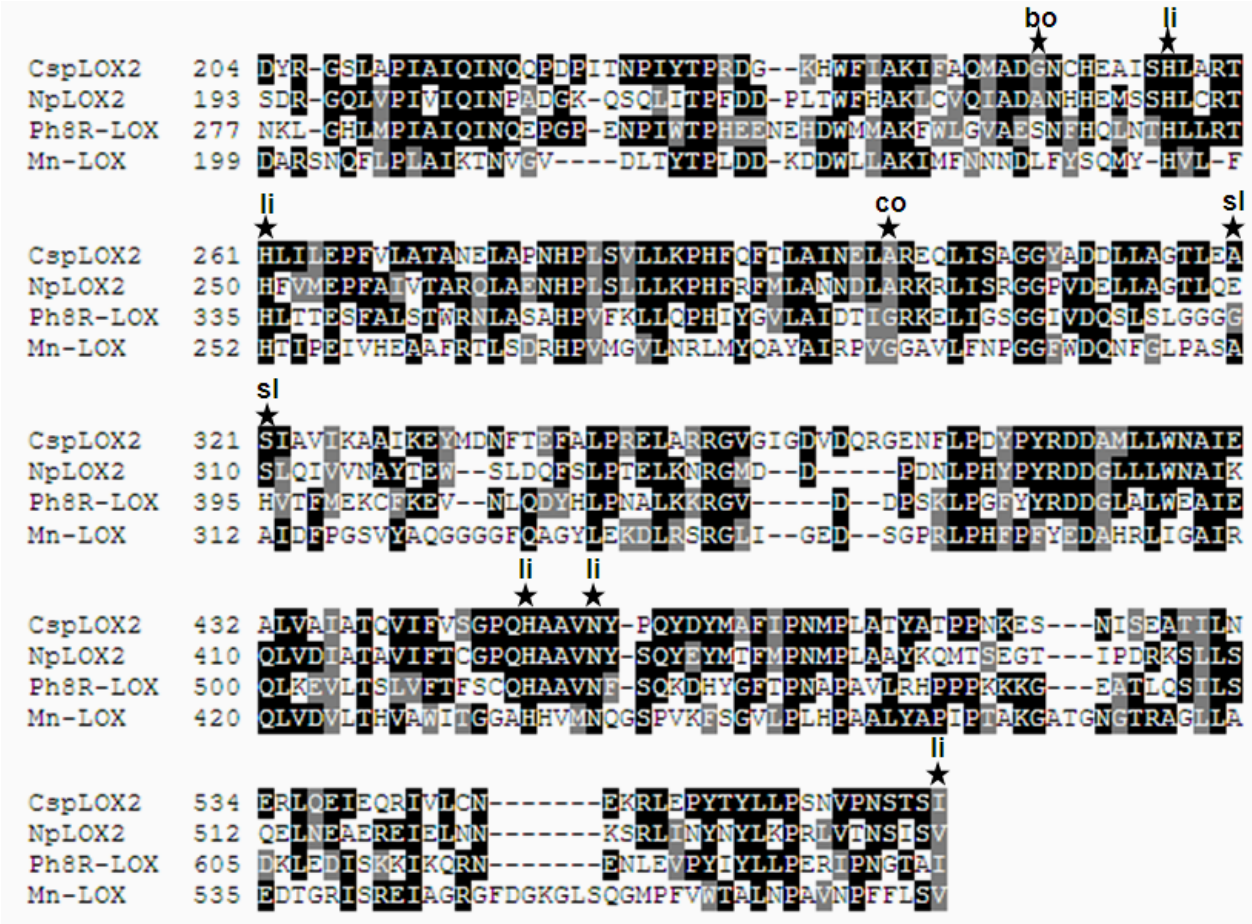

bo
li
li
co
sl
sl
li
li
li
CspLOX2 204 DYR-GSLAPIAIQINQQPDPITNPIYTPRDG--KHWFIAKIFAQMADGNCHEAISHLART
NpLOX2 193 SDR-GQLVPIVIQINPADGK-QSQLITPFDD-PLTWFHAKLCVQLADANHHEMSSHLCRT
Ph8R-LOX 277 NKL-GHLMPIAIQINQEPGP-ENPIWTPHEENEHDWMMAKFWLGVAESNFHQLNTHLLRT
Mn-LOX 199 DARSNQFLPLAIKTNVGV----DLTYTPLDD-KDDWLLAKIMFNNNDLFYSQMY-HVL-F
CspLOX2 261 HLILEPFVLATANELAPNHPLSVLLKPHFQFTLAINELAREQLISAGGYADDLLAGTLEA
NpLOX2 250 HFVMEPFAIVTARQLAENHPLSLLLKPHFRFMLANNDLARKRLISRGGPVDELLAGTLQE
Ph8R-LOX 335 HLTTESFALSTWRNLASAHPVFKLLQPHIYGVLAIDTIGRKELIGSGGIVDQSLSLGGGG
Mn-LOX 252 HTIPEIVHEAAFRTLSDRHPVMGVLNRLMYQAYAIRPVGGAVLFNPGGFWDQNFGLPASA
CspLOX2 321 SIAVIKAAIKEYMDNFTEFALPRELARRGVGIGDVDQRGENFLPDYPYRDDAMLLWNAIE
NpLOX2 310 SLQIVVNAYTEW--SLDQFSLPTELKNRGMD--D-----PDNLPHYPYRDDGLLLWNAIK
Ph8R-LOX 395 HVTFMEKCFKEV--NLQDYHLPNALKKRGV-----D--DPSKLPGFYYRDDGLALWEAIE
Mn-LOX 312 AIDFPGSVYAQGGGGFQAGYLEKDLRSRGLI--GED--SGPRLPHFPFYEDAHRLIGAIR
CspLOX2 432 ALVAIATQVIFVSGPQHAAVNY-PQYDYMAFIPNMPLATYATPENKES---NISEAIILN
NpLOX2 410 QLVDIATAVIFTCGPQHAAVNY-SQYEYMTFMPNMPLAAYKQMTSEGT---IPDRKSLLS
Ph8R-LOX 500 QLKEVLTSLVFTFSCQHAAVNF-SQKDHYGFTPNAPAVLRHPPPKKKG---EATLQSILS
Mn-LOX 420 QLVDVLTHVAWITGGAHHVMNQGSPVKFSGVLPLHPAALYAPIPTAKGATGNGTRAGLLA
CspLOX2 534 ERLQEIEQRIVLCN-------EKRLEPYTYLLPSNVPNSTSI
NpLOX2 512 QELNEAEREIELNN-------KSRLINYNYLKPRLVTNSISV
Ph8R-LOX 605 DKLEDISKKIKQRN-------ENLEVPYIYLLPERIPNGTAI
Mn-LOX 535 EDTGRISREIAGRGFDGKGLSQGMPFVWTALNPAVNPFFLSV

Figure 2

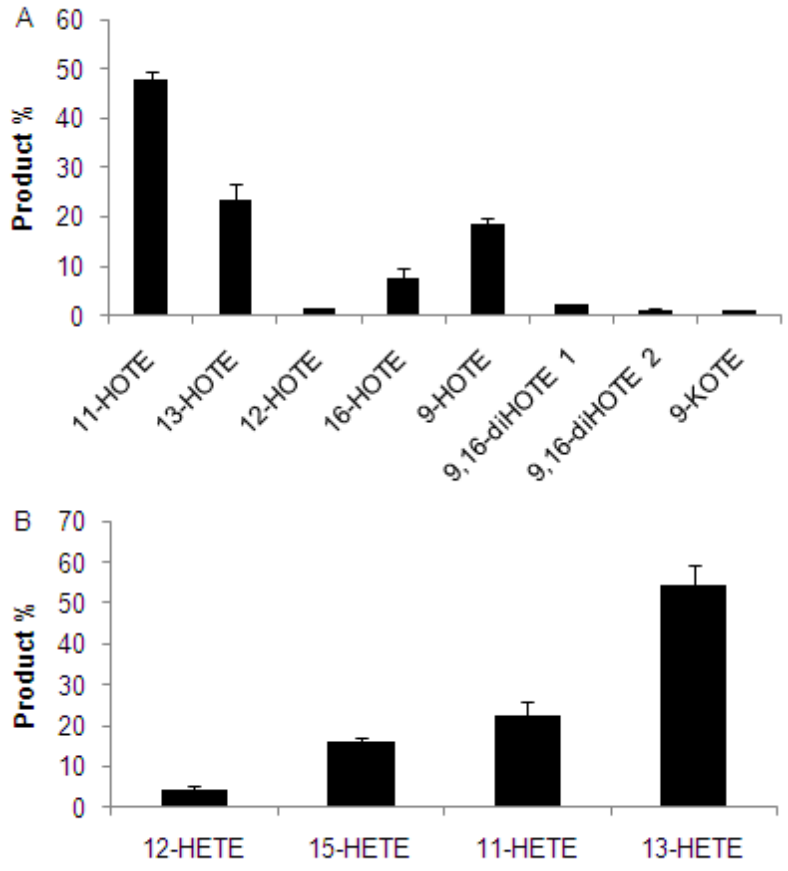
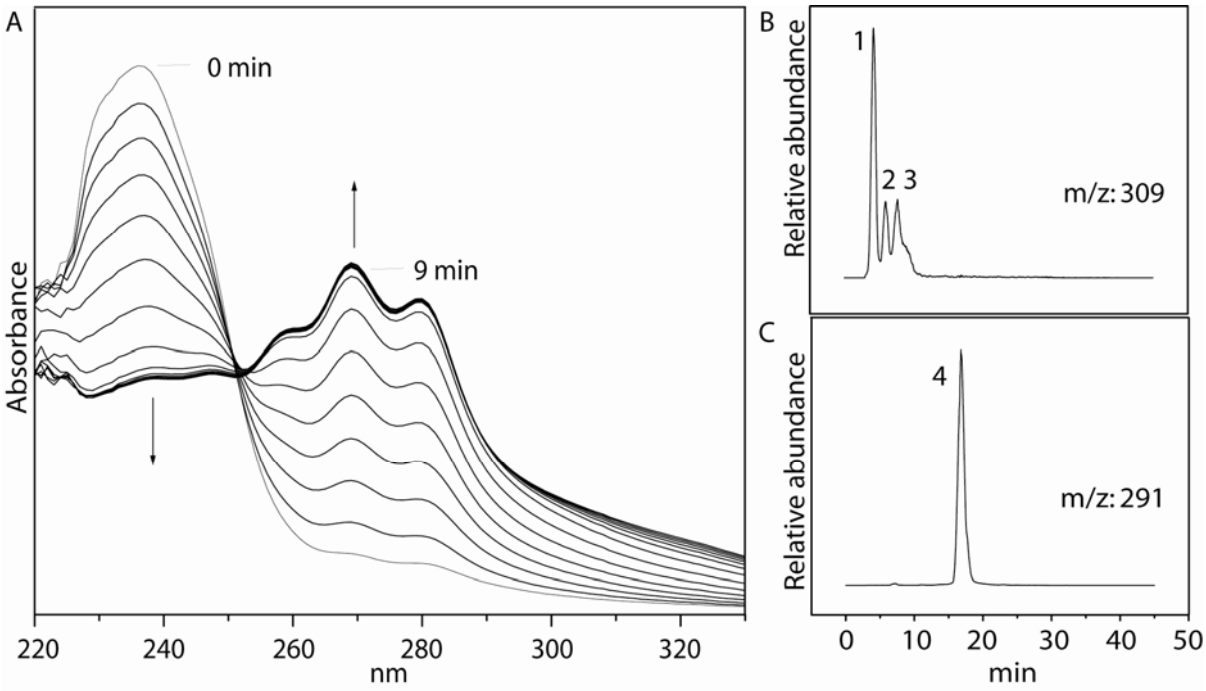

A
Product %
60
50
40
30
20
10
0
11-HOTE
13-HOTE
12-HOTE
16-HOTE
9-HOTE
9,16-diHOTE 1
9,16-diHOTE 2
9-KOTE
B
Product %
70
60
50
40
30
20
10
0
12-HETE
15-HETE
11-HETE
13-HETE

Figure 3


A
0 min
9 min
Absorbance
220
240
260
280
300
320
nm
B
1
2 3
m/z: 309
Relative abundance
C
4
m/z: 291
Relative abundance
0
10
20
30
40
50
min