Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Supplementary Information

α-Oxidative Decarboxylation of Fatty Acids Catalysed by Cytochrome P450 Peroxygenases Yielding Shorter-Alkyl-Chain Fatty Acids

Hiroki Onoda[a], Osami Shoji*[a, b], Kazuto Suzuki[a], Hiroshi Sugimoto[b, c], Yoshitsugu Shiro[d], and Yoshihito Watanabe*[e].

[1]Department of Chemistry, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-0802, Japan, [2]RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo-cho, Hyogo 679-5148, Japan, and [3]Resarch Center for Materials Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-0802, Japan

Address corresponding to:
Yoshihito Watanabe, Resarch Center for Materials Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-0802, Japan. Email: p47297a@nucc.cc.nagoya-u.ac.jp
Osami Shoji, Department of Chemistry, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-0802, Japan. Email: shoji.osami@a.mbox.nagoya-u.ac.jp

Material and methods

Materials

All chemical reagents were purchased from commercial sources and used without further purification. Tridecanoic acid (C_{13}), potassium dihydrogenphosphate, dipotassium phosphate, potassium chloride, 2-(*N*-morpholino)ethanesulfonic acid (MES), glycerol and dichloromethane were obteined Nacalai Tesque. α-hydroxymyristic acid ($C_{14}\alpha$-OH), hydrogen peroxide and IPTG were obtained from Wako. 99% *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TCMS) was obtained from TCI. Palmitic acid (C_{16}), myristic acid (C_{14}), β-hydroxymyristic acid ($C_{14}\beta$-OH), ω-hydroxylauric acid ($C_{12}\omega$-OH) and thrombin were obtained from Aldrich. Magnesium chloride ($MgCl_2$) was obtained from Kanto. PEG4000 was obtained from Katayama chemical. Imidazole was obtained from Kishida chemical. hydrogen peroxide labeled with 90% ^{18}O and water labeled with 99% ^{18}O were obtained from ICON. pBlueScript II SK (+)-*CYP152N1* was purchased from Biomatik.

The sequence of synthetic gene (*CYP152N1*) was optimized for protein expression in *E. coli*
ATGGGTAAAGTTATCCCGAAACAGGAAGGCCTGGATCACTCTGTGGACTTTCTGCG
CGAGGGCTATCTGTTTGTGGCAAACCGCCGTAAAtctTTCCAGTCTAACATCTTCGAG
AGCCGCCTGCTGGGCGAGCGTGTTATCTGCCTGGGTGGCGAAGAAGCTGCAGAGGT
GTTTTATGACGCGAACAAATTCACCCGTCAGGACGCGGCACCGAAACGTCTGCTGA
AAACCCTGTTCGGTGAAGGCGGTGTGCAGACCCTGGACGGTTCCGAACACACCCAC
CGCAAGCAGATGTTCATGAGCCTGATGACCAAAGAAAACATTGATCGCCTGCTGCG
CCTGACTTATCGTGAATGGAACCAGATCGAACGTATGGGTGAGGAAATCGTTCTGT
ACGACATCGCACAGGAGGTGCTGATGAAAGCAGTGTGCGAATGGTCTGGTGTTCCG
CTGGCTAAGGAAGAAGTGGGTAAGCGTACCGAAGAAATGCGTCTGCTGTTCGAATC
TGGTACCTCTCTGGGTCCGACCTATCTGCAGGGTCGCAAAGCGCGTTCTTCTGCGGA
AGTTTGGATTCGTCAGATGGTTAAAGAAGTTCGCTCTAACCGTCTGCTGCCGAACG
AACATACTGCGCTGTATGAATTCTCTTGGCACCGTGATGAGAGCGGTGAACTGCTG
CCGGAAGAGGTGGTGGCTGTTGAAGTGCTGAACATTCTGCGTCCGACTGTTGCTAT
CTCTGTGTACGTGCTGTTTACCGTGCTGGCGCTGCACCAGTTTCCGGACGTTAAGGA
ACAGGTTGAGCGTGGTGAGGTTTCTAAAACTGAGTTTGTGCAGGAAGTGCGCCGTT
TCTACCCGTTTTTCCCGGTTGCAGCAGCTCGTGTTAAAACCGATTTCGAATGGGATG

GCTATGCGTTCCCGGAAGGTACCCTGACTCTGCTGGACCTGTACGGTACTAACCAC
GACGTGTCTATTTGGACCGAACCGGATCGTTTCGACCCGTCTCGTTTCAAAGATTGG
AAAGAATCTCCGTTCAACTTCATCCCGCAGGGCGGTGGTGATGTTGATTTTGGCCAC
CGTTGCGCGGGTGAGCACGTTACCATCGCAATCCTGGCGCAGGTTATCGAACTGTT
CACCAAGGAGTATGCGTATACCGTTCCGCCGCAGGATCTGTCTTACTCTTTCGTTGA
CATGCCGTCCCTGCCGAAAAGCAAACTGCGTCTGACTCACCTGACCCGTAACCAGT
AA

Amino acid sequence of CYP152 family enzymes

>CYP152A4

gi|154685541|ref|YP_001420702.1| CypC [*Bacillus amyloliquefaciens* FZB42]

70% to CYP152A1 *Bacillus subtilis*

MDEQIPHEKGLDNSLALLRDGYVFVKNRAENYRSDVFRARLLGKTFICMSGAEAAKLF
YDTERFQREHALPKRVQKTLFGTGAIQAMDGERHKHRKLLFMSLMTPPRQKRLAEAV
AKQWKASAEMWEGSNRIVLFDEAKKVLCRAACEWAGVPLKDSEVKERAEDFTDMVD
AFGAVGPRHWKGRRARLKTEKWIEEVIEDVRSGKLQTPEGSALYEMAFHTELDGNRL
DSHMAAVELINVLRPIAAISYFITFSALALHDYPEYRGKLRSRDDQEAERFAHEVRRYY
PFAPFLGAVVKKDFVWKNCEFKKGASVMLDLYGTNHDSRLWENPNEFRPERFQGREE
NKFDFIPQGGGDPANGHRCPGEGMTVEVMKTSLAFLANEIEYDVPSQDLSFSLSRMPAL
PESGFVISDVRRI

>CYP152A5

gi|226948314|ref|YP_002803405.1| cytochrome P450 [*Clostridium botulinum* A2 str. Kyoto]

68% to CYP152A2 *Clostridium acetobutylicum*

MLIEEHIPKDKGIDNTFALLQEGYLFIRNRVDRYQSNLFETHLFGQKVICMTGEEAAKLF
YNEELFQRNGAAPKRIQKTLFGENAIQTMDDEEHIHRKHLFMSLMTPLSQKILAELVME
KWKSSIDKWEHDKEIVLFNEAKETLCQISCKWAGVPLHKSEIKNRAEDFNLMVNALGA
VGPQYWKGKMARSRAEKWISGIIQDVRSSRLDAEESTPLYAMAFHRDLDGNQMDTSM
AAIELINVLRPIVAISTFITFAALGLYEHPECKEKLISGGDSYVEMFAQEVRRYYPFAPFL
GARVRKDFILNECELKKGMLVLLDMYGTNHDSQIWEKPNEFYPERFKEWKGSLFDFIP
QGGGDPVKTHRCPGEGITMEIMKGSLDFLVNKIEFQVPKQDLSYSLIKIPTLPKSGFIMT
NIKRKF

>CYP152A6

gi|15614320|ref|NP_242623.1| fatty acid alpha hydroxylase [*Bacillus halodurans* C-125]

57% to CYP152A1 *Bacillus subtilis*

MKSNDPIPKDSPLDHTMNLMREGYEFLSHRMERFQTDLFETRVMGQKVLCIRGAEAV
KLFYDPERFKRHRATPKRIQKSLFGENAIQTMDDKAHLHRKQLFLSMMKPEDEQELAR
LTHETWRRVAEGWKKSRPIVLFDEAKRVLCQVACEWAEVPLKSTEIDRRAEDFHAMV
DAFGAVGPRHWRGRKGRRRTERWIQSIIHQVRTGSLQAREGSPLYKVSYHRELNGKLL
DERMAAIELINVLRPIVAIATFISFAAIALQEHPEWQERLKNGSNEEFHMFVQEVRRYYP
FAPLIGAKVRKSFTWKGVRFKKGRLVFLDMYGTNHDPKLWDEPDAFRPERFQERKDS
LYDFIPQGGGDPTKGHRCPGEGITVEVMKTTMDFLVNDIDYDVPDQDISYSLSRMPTRP
ESGYIMANIERKYEHA

>CYP152A7

gi|160881040|ref|YP_001560008.1| cytochrome P450 [*Clostridium phytofermentans* ISDg]

68% to CYP152A3 YP_004308874.1 *Clostridium lentocellum* DSM 5427

MIVKKQIPRDKCIDNTFTLLKEGYLFIQNRTERYSSDVFETRLLGKKAICISGKEAPKLFY
NPILMKKKGALPKRIQKTLFGVNAIQTMDGRRHLHRKKLFMTIMNQEEQDRLSKITTE
KWQEAISRWEGASRVVLYDEVNRILCQSVCEWAGVPLPASEVKCRAKDFSTMVNTFT
AIGPEYWKGKKARKRTEKWIRGIIEATRSGKLRPGRNSALHQIAYYKDLDGKLLSTQM
AAVELINVLRPVVAISTFITFTAVALYEHREYIKILRSSDENMREMFVQEVRRYYPFTPFL
GAITRKNFMWKGYNFKKGTLVILDVYGINHDARIWENPYKFRPERFSEKREHLFDFIPQ
GGGDPSKGHRCPGEGITIELMKLSVDFLVNKLEFKIPEQELRYSLIKIPSLPKSGFIMRKIK
TRTSM

>CYP152B3

gi|110347019|ref|YP_665837.1| fatty acid alpha hydroxylase, cytochrome P450 [*Chelativorans* sp. BNC1]

58% to CYP152B1 *Sphingomonas paucimobilis*

58% to CYP152B2 *Azotobacter vinelandii*

82% to gi|103488034 ref|YP_617595.1 [*Sphingopyxis alaskensis* RB2256]

MPRIPRDRSLDSTIALMGDPYRFISNRSRRYRSDLFETRLLLRKTICMTGPEAAQLFYDPS
RFVRTGAMPKAIQKTLLGVGGVQGLDDDAHRHRKQMFMSLMTPERIEQLVQLTAAE
WQIRVLKWGSMDEVVLYPELHLLLTRAVCAWAGVPLADSEVDARTKEIAALFDHAGA

VGLRHLWSRWARKQADRWIADIIDQIRSGRIRPPEQSAAHIIAWHRDLNGELLTPQIAA
VELINVIRPTVAVSVYMLFVAHALHAHPKIRERLQADEDSYSRRFVQEVRRYYPFFPAV
AARTRQAFDWNGYQFTAGRRVLLDLYGTNQDPRTWKRPEEFEPERFRHWDESPFNFIP
QGGGDHYVNHRCPGEWIAIELMKLTADFLSRSMSYEVPQQDLRIDWSRLPALPRSRFVI
RNVRES

>CYP152B4

gi|103488034|ref|YP_617595.1| fatty acid alpha hydroxylase, cytochrome P450 [*Sphingopyxis alaskensis* RB2256]

59% to CYP152B2 Azotobacter vinelandii

82% to gi|110347019|ref|YP_665837.1

MSRIPRDKSLDSTIALMGDPYRFISNRCRRYRSDLFETRLLLRKTICMTGPEAARLFYDQ
SRFARHGAMPKAIQKTLLGVGGVQGLDEGAHRHRKQMFMSLMTPEGIEKLVRLTSAE
WQIRVRKWASMNAVVLYPELHALLTRAACAWAGVPLADSEVEPRTRQVTALFDHAG
SIGLRHLWSRRARKRADSWAADIVEQIRSGRLRAPEHSAAHVVAWHRDFSGALLTPQV
AAVELINVIRPTVAVSVYMIFVAHALHTHPQVREKLQAGDEDYAECFVQEVRRYYPFF
PAVAAQTRLAFEWNGYQFPAGRRILLDLYGTNQDPRTWERPEDFEPERFGRRDGSPFN
FIPQGGGDHYFDHRCPGEWIAIELMKLTADFLTRRMSYEVPGQDLRIEWSRLPALPRSR
FVLSNVRECQELHLQRTRWRRR

>CYP152B5

gi|256826347|ref|YP_003150307.1| cytochrome P450 [*Kytococcus sedentarius* DSM 20547]

52% to CYP152B1 *Sphingomonas paucimobilis*

MTPSNIKRADGTIALLRDPYRCISRRAAELGEDIFETRLLLRRTTCMTGAEAAAVFYDPS
RFQRAGAAPPPLQKTLFGQGGVQGLDGENHRQRKAMFLQIVQPDRVEALAEAVTREW
QRAVDDWIGQGRTRLYPELQLLLTRAVCAWAGVPLPAAEVNTRTRQLSALFDQAGHV
GVGHLRSRAARKAADRWAADIIGQVRAGKLDPPASSAAYVIAHHRERDGRPMAPRVA
GVELLNVLRPTVATAVYITFVAHALDAQPAWKERLALGDGHEDLAFVEEVRRHYPFFP
AVAAIVREEFVWRGHRFPRGRRVLLDLYGTNHDSRIWPDPQRFDPDRFLGEEPDPFAFV
PQGGGDPAVHHRCPGEPVSTRLMTVALDQMVRNMTYTALAPSAAVDFGRLPALPTDG
YPIRLLARDTPA

>CYP152N1 (P450$_{Ex\alpha}$)

gi|229918020|ref|YP_002886666.1| cytochrome P450 [Exiguobacterium sp. AT1b]

48% to CYP152K1 *Bacillus clausii* KSM-K16 BAD65574

44% to CYP152H1 ZP_10970070.1 *Sporolactobacillus vineae* DSM 21990

MGKVIPKQEGLDHSVDFLREGYLFVANRRKSFQSNIFESRLLGERVICLGGEEAAEVFY
DANKFTRQDAAPKRLLKTLFGEGGVQTLDGSEHTHRKQMFMSLMTKENIDRLLRLTY
REWNQIERMGEEIVLYDIAQEVLMKAVCEWSGVPLAKEEVGKRTEEMRLLFESGTSLG
PTYLQGRKARSSAEVWIRQMVKEVRSNRLLPNEHTALYEFSWHRDESGELLPEEVVAV
EVLNILRPTVAISVYVLFTVLALHQFPDVKEQVERGEVSKTEFVQEVRRFYPFFPVAAA
RVKTDFEWDGYAFPEGTLTLLDLYGTNHDVSIWTEPDRFDPSRFKDWKESPFNFIPQGG
GDVDFGHRCAGEHVTIAILAQVIELFTKEYAYTVPPQDLSYSFVDMPSLPKSKLRLTHL
TRNQ

>CYP152P1 (CYP-MP)

gi|188580553|ref|YP_001923998.1| protein CypC [*Methylobacterium populi* BJ001]

60% to gi|170742430 ref|YP_001771085.1 [*Methylobacterium* sp. 4-46]

45% to CYP152A1 *Bacillus subtilis*

45% to CYP152G1 ZP_16052477.1 *Rhodopirellula baltica* SH28

MPAAIATHRFRKARTLPREPAPDSTLALLREGYGFIRNRCRRHDSDLFAARLLLSPVICM
SGAEAARHFYDGHRFTRRHALPPTSFALIQDHGSVMVLDGAAHLARKAMFLSLVGEE
ALQRLAGLAERHWREAVSGWARKDTVVLLDEAHRVLTAAVCEWVGLPLGPTEVDAR
AREFAAMIDGTGAVGPRNWRGHLYRARTERWVRKVIDEIRSGRRDVPPGAARTIAEHQ
DADGQRLDRTVAGVELINVLRPTVANARYIVFAAMALHDHPHQRAALADGGEAAERF
TDEVRRFYPFIPFIGGRVRAPFHFGGHDFREGEWVLMDLYGTNRDPRLWHEPERFDPD
RFARETIDPFNMVSHGAGSARDGHRCPGEGITRILLRTLSRQLAATRYTVPPQDLTLDL
AHVPARPRSGFVMRAVHAP

>CYP152P2

gi|170742430|ref|YP_001771085.1| cytochrome P450 [Methylobacterium sp. 4-46]

60% to CYP152P1 *Methylobacterium populi*

47% to CYP152H1 ZP_10970070.1 *Sporolactobacillus vineae* DSM 21990

MFAIPRDPAPDSTLALLREGYGFIPGRCRNFGSDLFTTRLMLTNVVCMTGADAAAQFY
APDRFTRRGALPRISFTLIQDNGSVMVMDGEAHRRRKAMFLSLMSPEALQRLADLTTQ
AWRARMRRWAAQETIPLFDEAHVPFCAAVCAWAGLPLNEAEAEERSREFLAMIEGTG

SIGPRNWRGHLLRARAERWMRDAIRQIREGSRAVPEGSAAHVIAHHRDAEGRLLDVRT
SAVELINILRPTVANARYVVFAAHALHCYPESREALQAAEGVEPFVQEVRRFYPFIPFIG
GRALQTVEFHGHRFAVGDWVLMDLYGTNRDPNRWGQPERFIPDRFAQWDHDPNGFIP
QGGGAYETGHRCPGEWITIEQMRAVVPLLAREMRYVVPEQDLTIDLGRIPAMPKSRFVI
TKVAG

>CYP152Q1

gi|170783544|ref|YP_001742037.1| putative fatty acid beta hydroxylase [*Arthrobacter* sp. AK-1]

44% to CYP152G1 ZP_16052477.1 *Rhodopirellula baltica* SH28

47% to CYP152C1 *Rhodobacter sphaeroides*

MTVQLPDSSLALLREGYTFISSRCDRLGTDLFRTTLILRPVVCLRGAEAAEFFYGGGRFG
RKAAMPRSAQHLLQDAGSVQSLQGSAHRRRKQLFLDLMTNESVERLGRAFDTEWHSA
RERWQDSGEVVLHDEVRRVLTATACHWAGVSADRATVSRRARELSLMIDKAGAVGP
VNWYARWRRRSTEKWAGECISSIRRNGPAAASGSPAAVIAFHTDEHGNPLSAEVAAVE
LLNLLRPIVAVSRFMVFAAVALQQHPEWNDIVRKGQDADLDCFAQEVRRYYPFFPFVG
GTARETLEWKGHTFKTGQWALFDLYGTNHDGRLWKDPESFNPARFRVWRPDPHTLVP
QGAGDAAVGHRCPGEDITVDLIRRATRALAAESGISVPAQDLSIDLTRMPALPRSGFILS
VGRASD

>CYP152R1

gi|146283602|ref|YP_001173755.1| fatty acid alpha hydroxylase [*Pseudomonas stutzeri* A1501]

47% to CYP152E1 gi|126657198| ZP_01728364 *Cyanothece* sp. CCY0110

46% to CYP152G1 ZP_16052477.1 *Rhodopirellula baltica* SH28

MTDIPRDDHLESSLALLGEGYPFIRDRCQRLHSNVFQARLLMQNTICLSGEQAARLFYD
ERHFQRAQAMPRMLKKTLLGQGGVQGLDGEAHRHRKRMFLQLLDAAAVDELVGLTE
RSWRQAIGQWQAQGEVQLLGELQMLLTDSVCRWAGVPLPSAERELRRDQLAQMIDG
AGGIGPRHLAARKARREAEAWTQHLIEQVRAGELQGDPTRALMVVAHHRDLDGKPLD
SRIAAVELLNLLRPTVAVAYFITYAALELLAHPHWCERLRAEDELLEPFAQEVRRLHAF
FPFTAARVRDGFDWQGHHFPAGTRVMLDLWGTNREASRWTDPEAFQPERFVDWPGD
AFGFVTQGGGDPAQGHRCPGERLAIELLKLALRMLTREMDYAVPAQDLRIDLSRMPAK
PESGLLISDVRPRAS

>CYP152S1

gi|91775795|ref|YP_545551.1| fatty acid beta hydroxylase (cytochrome P450) [*Methylobacillus flagellatus* KT]

49% to CYP152H1 ZP_10970070.1 *Sporolactobacillus vineae* DSM 21990

49% to CYP152E1 gi|126657198| ZP_01728364 *Cyanothece* sp. CCY0110

MARPPLESTYALWKKGYVFIDSTCRALGVKAFRTRLMMRPAICMQGEAAARLFYDNE
KFMRAGAMPARAKKTLVGEGGVQGMDGHAHRHRKQMFTRELMSPERIEHLVCLHRG
HWKEAIQAWKPGSEIVLFREAQKLLCKSVCAWAGVPLTGHELDRRAREFGHMIDSGA
KLGPHHWRGRWARKSCERWLGQLIEDIRHYGEQGQAGSILQSIAWHRDEHGQRLPLQ
VAAVELINILRPTVAIAQLAVFCALALHSFPACRQKLQQGDEDYLGWFVLEVRRFYPFF
PFLAAVVREDFRWQNLEFKRGQRVLLDIYGTHRDPALWQDPAQFIPERFKDWDGNSFG
LIANGGATYEYHHRCPGEWITIALMKAITRMLVHEIEYTVPAQDLRLDLSRIPALPESGF
IIQPVITPRYLEKKHANS

Supplemental Figures

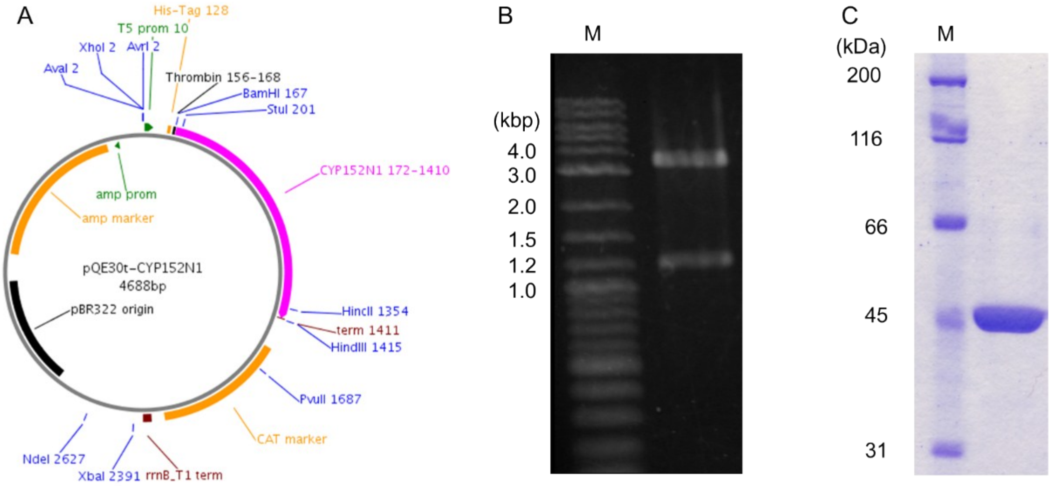

Fig. S1. A) The construction of transformation plasmid pQE30t-*CYP152N1* and B) Agarose gel electrophoresis of pQE30t-CYP152N1 treated by *Bam*HI and *Hin*dIII C) SDS-PAGE of purified CYP152N1

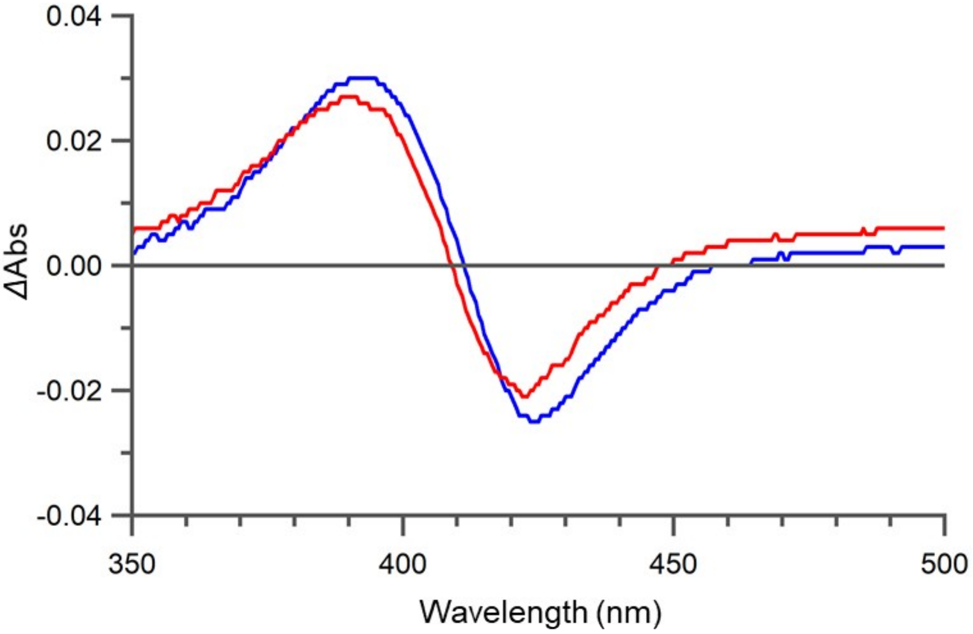

Fig. S2 Difference spectra between CYP152N1 (6.2 μM) without substrate and CYP152N1 with 50 μM of Myristic acid (Red), 10 μM Palmitic acid (Blue).

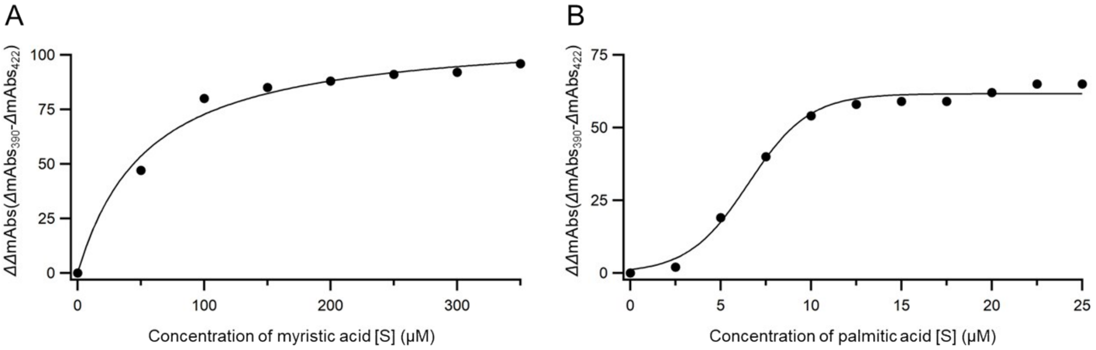

Fig. S3 Difference absorbance ($\Delta\Delta$mAbs$_{390\text{-}422}$) plot between CYP152N1 without substrate and CYP152N1 with myristic acid (A) and palmitic acid (B).

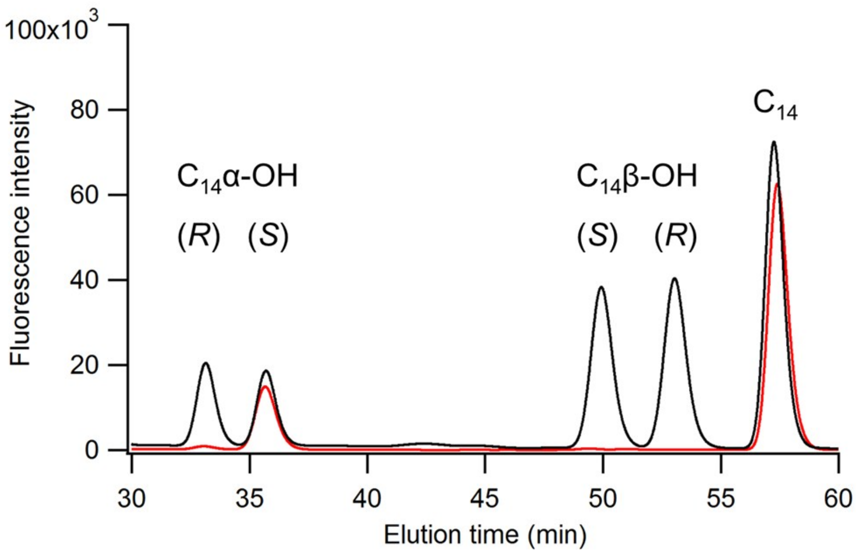

Fig. S4 HPLC chart equipped with Chiralpak AD-RH column and fluorescence detector for myristic acid hydroxylation catalyzed by CYP152N1 (Red). The retention time of α-hydroxymyristic acid (C14α-OH), β-hydroxymyristic acid (C14β-OH) and myristic acid (C14) were detected by 1:2:2 authentic samples (Black). The enantio-selectivity were determined by the product catalyzed by CYP152A1 and CYP152B18. Retention time: C14α-(R)-OH (33 min), C14α-(S)-OH (36 min), C14β-(R)-OH (50 min), C14β-(S)-OH (53 min), C14 (57 min).

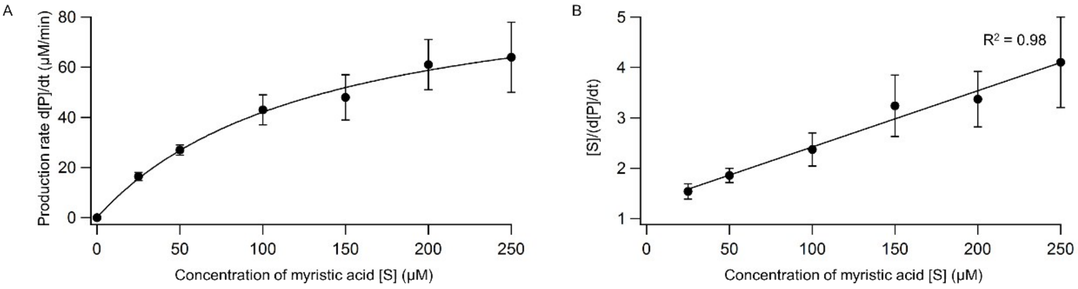

Fig. S5 Michaelis-Menten plot (A) and Hanes-Woolf plot (B) of myristic acid hydroxylation catalyzed by CYP152N1 under the various concentration of myristic acid.

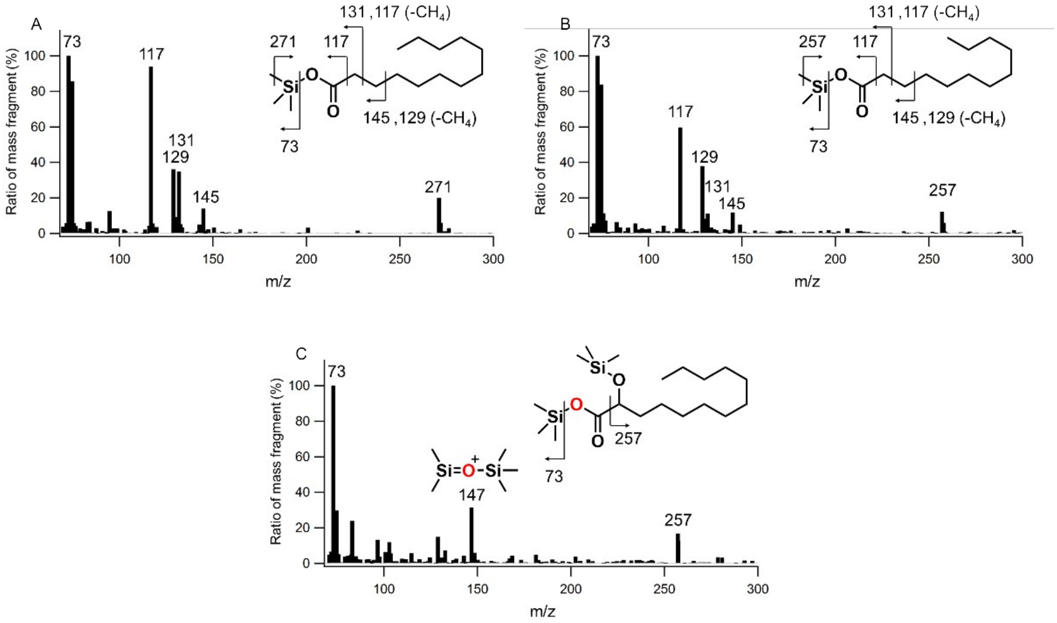

Fig. S6 EI-Mass Fragment pattern at the peak of Fig.4A on 11.7 min (A, tridecanoic acid), 10.7 min (B, lauric acid), and 13.4 min (C, α-hydroxymyristic acid).

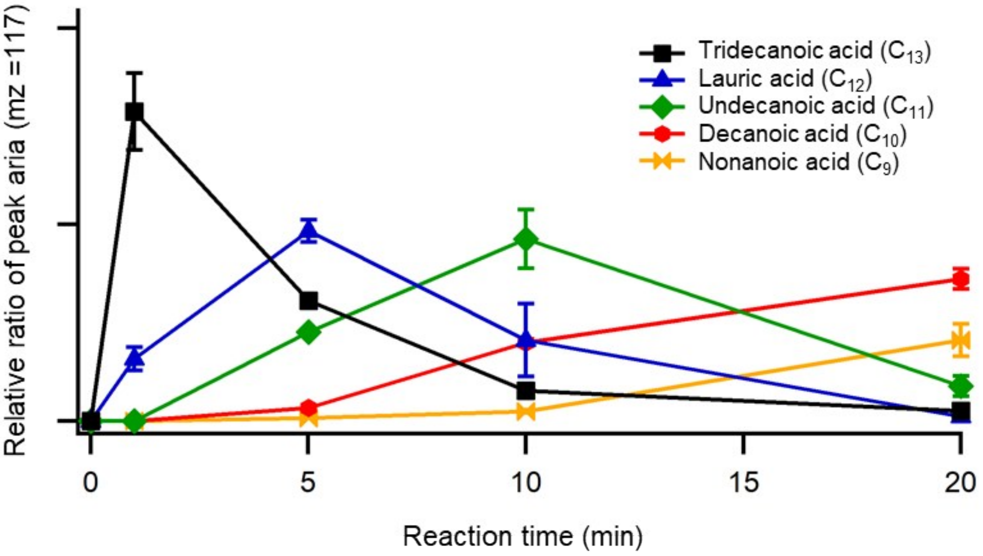

Fig. S7 Transition of fatty acid ratio under the myristic acid oxidation catalyzed by 1 µM CYP152N1 in the presence of 5 mM H_2O_2 and 100 µM myristic acid.

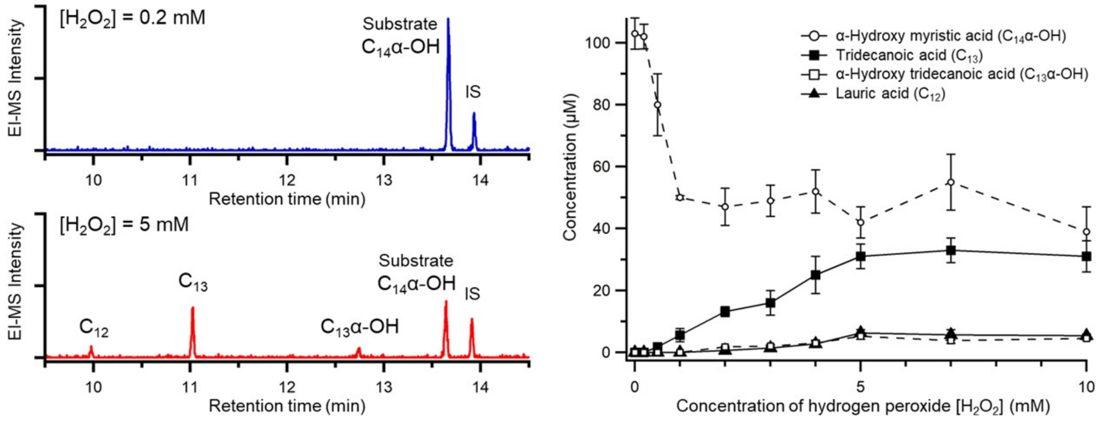

Fig. S8 GC-MS chart (MIC m/z = 117, 147) of α-hydroxy myristic acid oxidation in the presence of 200 µM (A) or 5 mM (B) H_2O_2. Retention time of TMS derivatives: C_{12} (10.0 min), C_{13} (11.0 min), C_{14} (12.0 min), $C_{13}\alpha$-OH (12.7 min), $C_{14}\alpha$-OH (13.7 min), $C_{14}\beta$-OH (13.8 min) C_{16} (13.9 min)

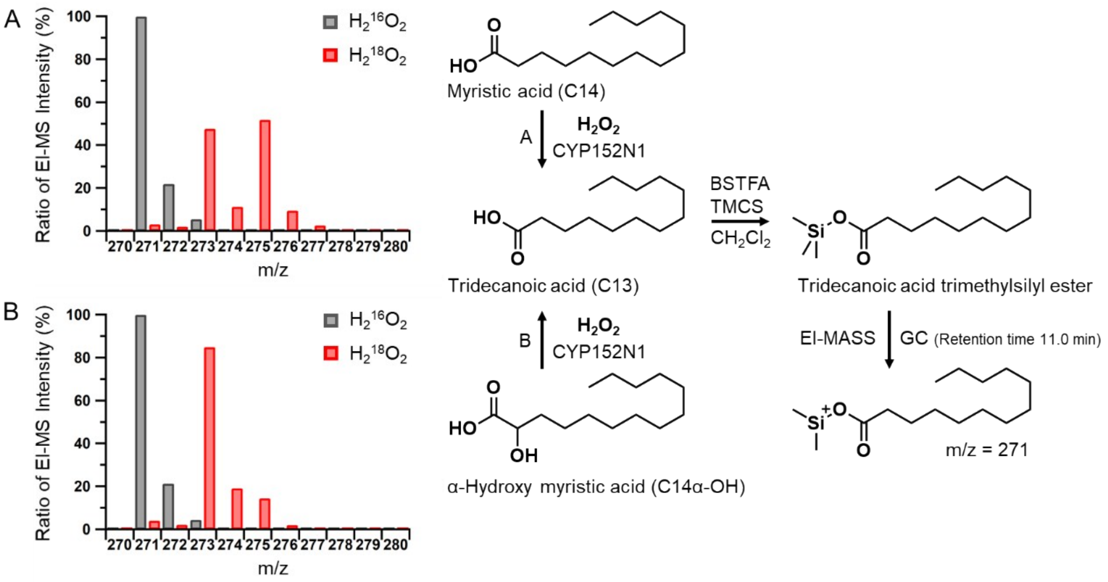

Fig. S9 Ratio of EI-Mass Fragment Intensity (m/z =270-280) at GC retention time of Tridecanoic acid trimethylsilyl ester (11.0 min) produced from (A) myristic acid and (B) α-hydroxy myristic acid using $H_2{}^{16}O_2$ (black, Fig 2 or Fig S8) or $H_2{}^{18}O_2$ (red).

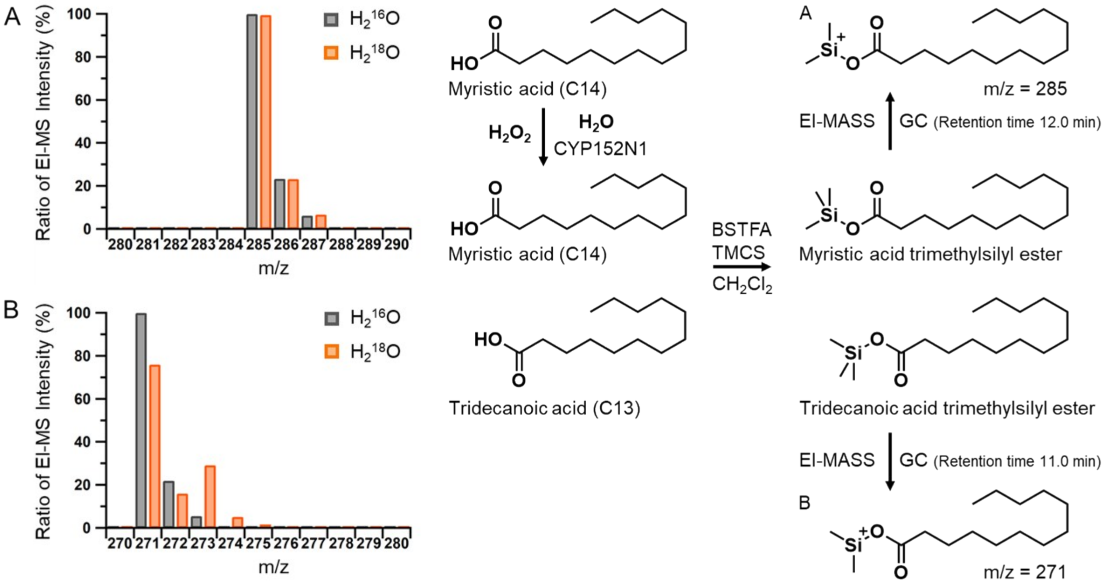

Fig. S10 Ratio of EI-Mass Fragment Intensity (m/z =280-290 or 270-280) of the reaction solution resulted at GC retention time of myristic acid (A, 12.0 min) or tridecanoic acid (B, 11.0 min) produced by myristic acid using $H_2{}^{16}O$ (black, Fig. 2) or

$H_2{}^{18}O$ (orange).

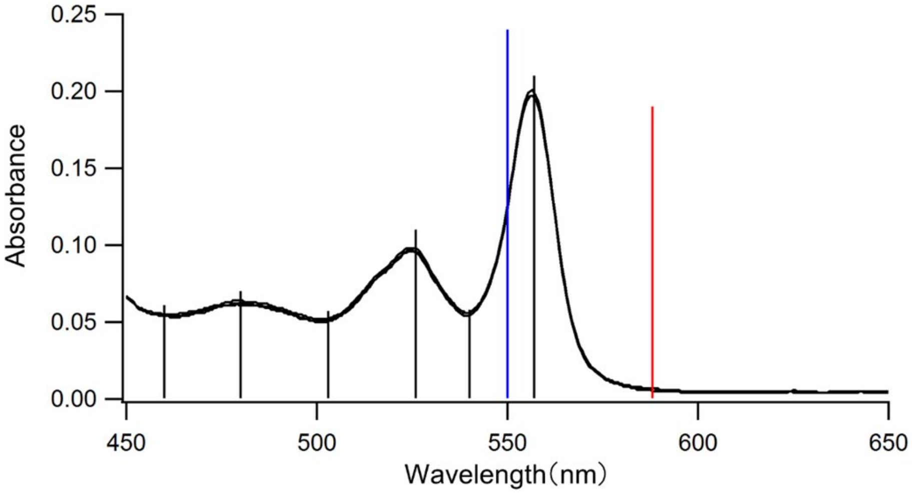

Fig. S11 Pyridine hemochrome assay of CYP152N1. Black stick was shown as maximum absorption and minimum absorption of heme *b*. The maximum absorption of heme *a* and heme *c* was shown as red stick and blue stick. Stick length show as relative extinction coefficient (ε) in contrast with ε_{557nm} of heme *b*.

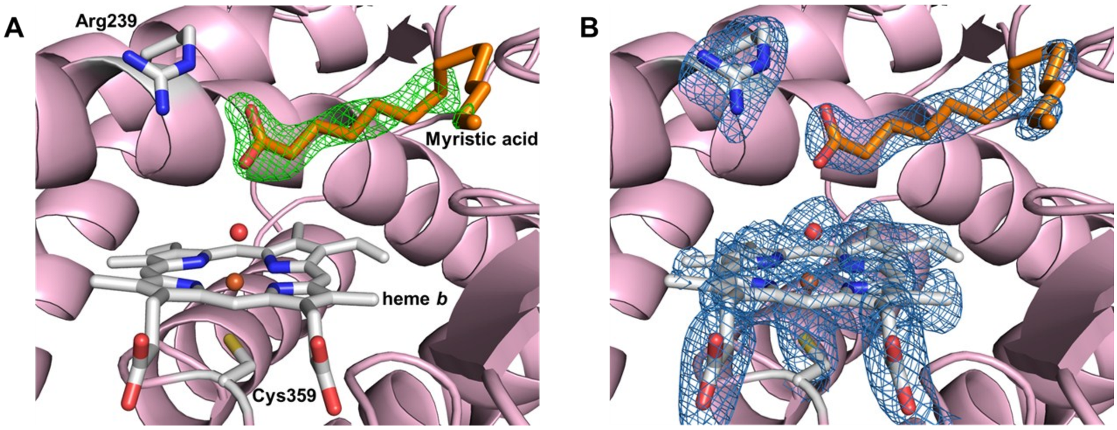

Fig. S12 F_o-F_c omit map for the myristic acid was shown as green mesh contoured at 2.5σ level (A). $2F_o$-F_c electron density maps of myristic acid and heme were shown as blue mesh at the 1.0σ level (B). Heme *b*, Arg239, and Cys359 were shown as white stick. Myristic acid was shown as orange stick. Iron cation and sixth ligand water were shown as sphere. The electron density of alkyl chain of fatty acid was unclear owing to the high flexibility.

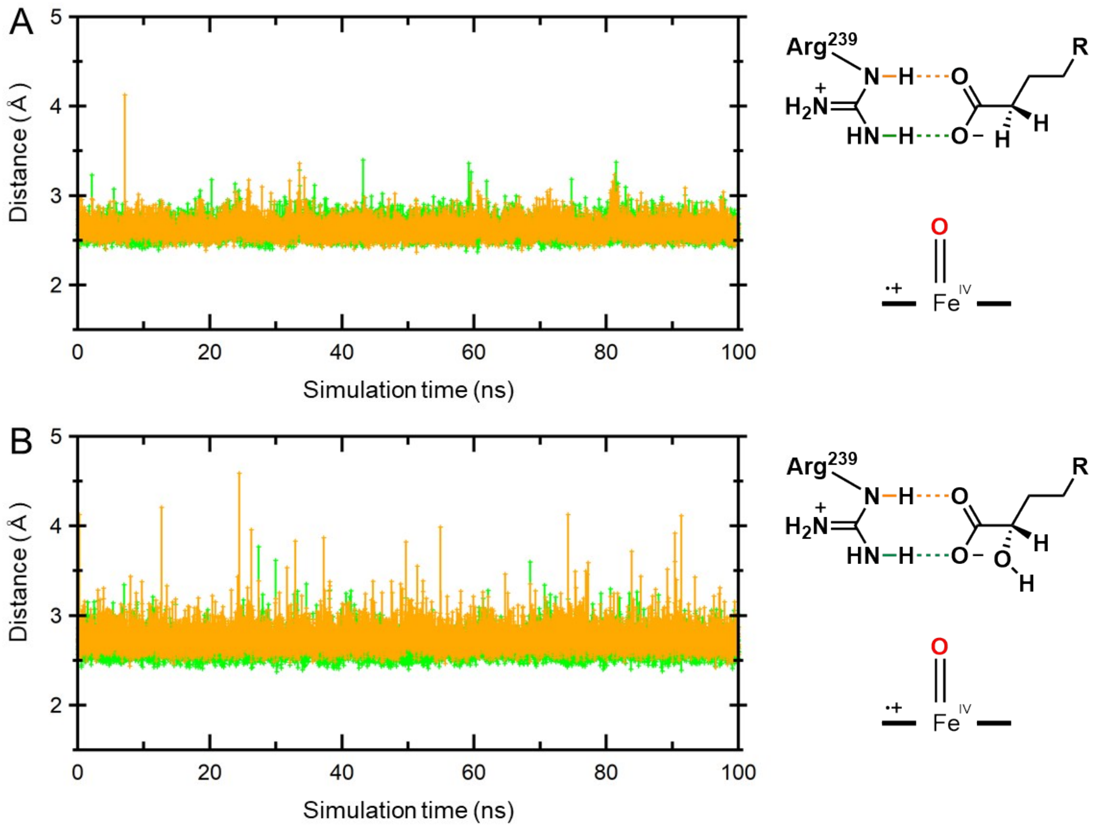

Fig. S13 100 ns distributions of N-O distance between Arginene239 and Carboxylate. The plots of distance were shown as yellow or green color.

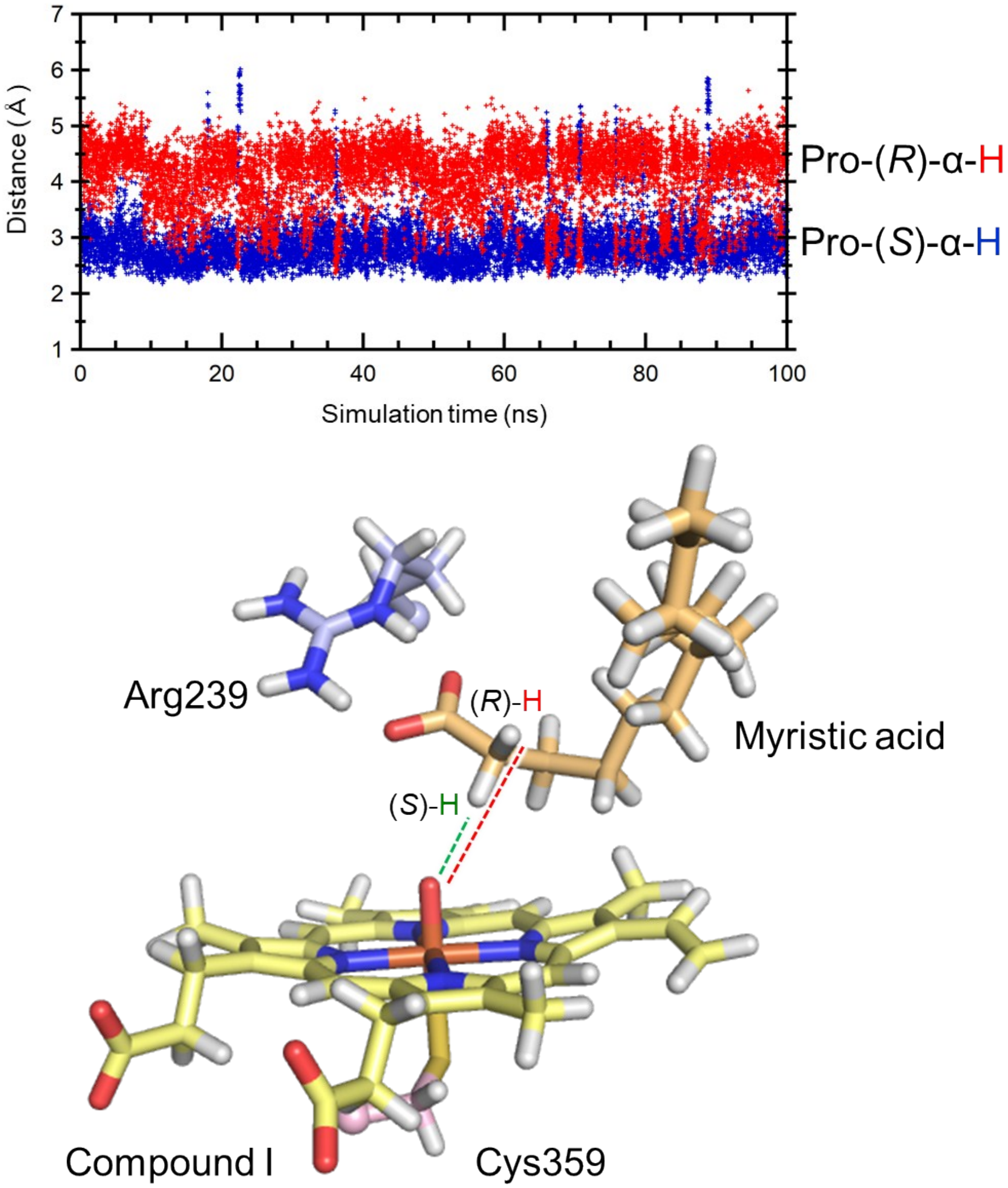

Fig. S14 100 ns distributions of O-H distance between oxygen atom of Compound I and hydrogen atom. The plots of hydrogen atom of myristic acid at (S)-α-hydrogen atom were shown as blue color. The plots of (R)-α-hydrogen atom were shown as red color.