Supporting Information

Probing fatty acid metabolism in bacteria, cyanobacteria, green microalgae and diatoms with natural and unnatural fatty acids

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SI Figures and Tables

Table S1 - Fatty acid synthases of various organisms

Protein	*E. coli*	*ref*	*Synechocystis sp PCC6803**	*ref*	*Chlamydomonas reinhardtii*	*ref*	*Loc*	*Pred algo*	*Thalassiosira pseudonana*	*Predalgo*	*ref*	*Arabidopsis thaliana*	*ref*	*Trypanosoma brucei*	*ref*	*Homo sapiens*	*ref*
ACP	AcpP	[1]	WP_010871942.1	[2]	Q6UKY5	[3]	C	C	XP_002289159.1 XP_002291006.1	O O	-	At3g05020 At1g54580 At1g54630 At4g25050 At5g27200	[4]	Tbg972.3.490	-	Type I FAS	[5]
MCAT	FabD	[6]	WP_010871898.1	-	A8HP61 (XP_001689862.1)	[7]	C	C	XP_002290601.1	C	-	AT2G30200.1 or AT2G30200.2	-	Tbg972.9.7190	-	Type I FAS	-
KS3	FabH	[8]	WP_010872643.1, WP_015390150.1 (same protein!)	-	A8JHL7 (XP_001703101.1)	[7]	C	O/C	XP_002295320.1	SP	-	At1g62640	[9]	no hits using Pf	-	Not present	-
KS2	fabF	[10]	~~WP_010872715.1~~ WP_010871941.1	[11]	A8JCK1 (XP_001700152.1)	[7]	C	C	XP_002290056.1 XP_002291090.1 XP_002295282.1	O/C O O		At1g74960	[12]	Tbg.972.2.2150	-	Type I FAS	-
KS1	FabB	[13]	Not present	-	A8JEF7 (XP_001701199.1)	[7]	C	C	XP_002290056.1 XP_002295282.1	O O	-	At5g46290	[14]	one FabB/F homolog	-	Type I FAS	-

								XP_00229109 0.1	O								
KR	FabG	[15]	WP_0108 72244.1 ~~WP_0111 53616.1~~	-	Q84X75	[7]	C	C	XP_00228766 7.1 (maybe XP_00228853 4.1 and more)	M C	-	At1g24360 AT1G62610.4 AT1G63380.1 AT3G46170.1 AT3G55290.1 AT3G55310.1	[16]	Tbg972.10.1437 0 Tbg.972.2.3240 Tbg972.5.1680	-	Type I FAS	-
DH DH	FabA FabZ	[17] [17]	No hits WP_0108 72534.1	- -	No hits A8IX17 (XP_00169 3164.1)	[7]	C	O	No hits XP_00229117 9.1	O	-	No hits At5g10160 (NP_196578.1) AT2G22230.1 AT5G60340.1		- Tbg972.11.1277 0	- -	- Type I FAS	- -
ER	FabI	[18]	WP_0144 07072.1	-	A8JFI7 (XP_00170 1585.1)	[7]	C	O	XP_00228823 6.1	O	-	At2g05990 AT3G45770.1	[19]	no hits using Pf	-	Type I FAS	-
TE	none	-	none	-	A8HY17	[7]	C	C	No hits	-	-	AT3G25110.1 (FatA1) AT4G13050.1 (FatA2) AT1G08510.1 (FatB)	[20]	none	-	Type I FAS	-
Mitochondrial FAS																	

ACP	-		-		Q6UKY4	[7]	M	M	XP_002291006.1 XP_002289159.1		At2g44620 At1g65290 At5g47630		AAX69898 (Tb927.3.860)	[21]	THC 141079	[22]
MCAT	-		-		XP_001701756.1		M	O	EJK44401	C	AT2G30200.1 or AT2G30200.2		?		MCT1	
KS3	-		-		XP_001695929		M	SP/M	one KS?		only one KS		one KS			
KS2	-		-		XP_001690385		M	O/M	XP_002290056.1 and XP_002291090.1		only one KS		AAX79622	[21]	CEM1	
KS1	-		-		same hit KS2		M		XP_002295282.1		BAB91181.1	[23]	one KS			
KR	-		-		many hits		M		many hits		many hits		AAX79999 AAX69287 AAX69289	[21] [21] [21]	Hs17b-HSD8 and HsCBR4 (yeast: OAR1)	[24]
DH	-		-		XP_001703153.1 (MaoC like) XP_001691219.1		M M	C O	XP_002292033.1 XP_002291943.1	O	NP_001190580.1 NP_177742.2		Q580H9_9TRYP	[25]	HTD1 ot HTD2	

					XP_001695861.1		M	M	XP_002286573.1		NP_565528.1 (AAM64548.1)			
ER	-		-		several hits		M		several hits		several hits several hits		XP_827075 AAX79977	[21] [21]	ETR1	[26]
TE	-		-		none				none		none		none		none	
Accessory enzymes																
AasS	Aas**	[27]	Slr1609	[28]	*XP_001702947.1* *XP_001693692.1* *XP_001691289.1* *XP_001690836.1*				*XP_002291517.1* *XP_002295745.1* *XP_002289883.1* *XP_002295107.1*		AAE15	[29]	no hits		no hits	
PPTase	AcpS	[30]	WP_010873553.1	[31]	XP_001700873.1 (PPTC1) XP_001689489.1 (PPTC2)	[32]			CrPPTases do not give any hits. Maybe: XP_002297067		gi22330990 gi2947064	[32]	no hits using Pf PPTase, but present in *T. congolese*	[32]	AASD HPPT	[33]
AcpH	AcpH	[34]	none		none				none		none		none		none	
KCS	-		-		XP_001697210 ?				XP_002289421.1	[35]	n.d		n.d.		n.d.	
Elongases	-		-		none				XP_002288481.1 XP_002293395.1 XP_002291938.1	[35]	n.d.		n.d.		n.d.	

Table S1 – Fatty acid synthases of various organisms. Genes were putatively annotated by mining the literature (see references) and by psi-blasting known enzymes against the specific organisms in the NCBI database. Putative localization prediction using Predalgo {Tardif, 2012 #154} is included for the algae (C, chloroplast, N, nuclear, M, mitochondria and O or SP, other/secretory) *) Genes were identified by psi-blasting the known *E. coli* enzymes using the cyanobacterial genome. **) Acyl acyl carrier protein synthetase is a unique bifunctional membrane bound enzyme in *E. coli*. The enzymes from *V. harveyii*, *Synechocystis* sp PCC 6803 or *A. thaliana* are soluble single domain enzymes.

Table S2 – Fatty acid desaturases of *Synechocystis* sp PCC 6803

Name	Annotated protein name (Uniprot)	Target	Redox partner	Reference(s)
DesA	Slr1350	D12	Ferredoxin*	[36]
DesB	Q79EF1	D15	Ferredoxin	[37]
DesC	F7UT40	D9	Ferredoxin	[38]
DesD	F7URB7	D6	Ferredoxin	[39]

Table S2 – Fatty acid desaturases of *Synechocystis* sp PCC 6803. These desaturases have been well described in the literature. *cyanobacterial desaturases utilize ferredoxin as electron-donor, although cytochrome b5 can also be the donor in an engineered system.[40]

Table S3 – Fatty acid desaturases of *C. reinhardtii*

	K/U***	Accession (Uniprot)	Accession (Ncbi)	Annotated protein name	Target	Match tree Figure 5	Predalgo[41]	Redox partner	Comments / FAD	Reference(s)
1	K	Q2HWK7	XP_001698534	ω13 fatty acid desaturase	ω13 d5	Tree: close to d4, d5, d6 desaturases from apicomplexan and bacteria	O	Contains CytB5 domain	CrDES, front-end desaturase	[42]
2	K	A8IR24	XP_001691669.1	Microsomal Δ12 fatty acid desaturase	d12	Tree: close to 200 plant d12 desaturases	O		FAD2 Blast: many delta-12 microsomal desa in algae and plants	[43, 44]
3	K	O48663	XP_001693068	Chloroplast ω6 desaturase	ω6 d12	Tree: close to 200 plant omega 6 desaturases	C		DES6. Omega-6-FAD. FAD6. 18:1d9 > 18:1d9,12	[45]
4	U	A8IUT7	XP_001692663.1	Δ9-ACP desaturase-like protein	d9	Tree: close to 280 plant d9-ACP desaturases	O	ferredoxin		
5	U	A8JEN7	XP_001701272	Fatty acid desaturase	d7? d9?	Tree: close to 200 cyano and plant d9 and d7 desaturases	C		FAD5c, d7 or d9? Stearoyl-CoA 9-desaturase	
6	U	A1E5M5	ABL09485	ω3 fatty acid desaturase	ω3	Tree: close to 200 plant chloroplast	C			

					d15	omega 3 desaturases				
7	U	A8HMC4	XP_001689663	Chloroplast glycerolipid omega-3-fatty acid desaturase	ω3 d15	Tree: close to 200 plant chloroplast omega 3 desaturases	C		FAD7, chloroplast	
8	U	A8JEN2	XP_001701270	Fatty acid desaturase	d7? d9?	Tree: close to 200 cyano and plant d9 and d7 desaturases	O		FAD5d = d7, matches d9, looks like C6ZE81	
9	U	A8J015	XP_001694618	MGDG specific C16 d7 desaturase	d7	Tree: close to 200 cyano and plant d9 and d7 desaturases	O		FAD5a = d7	
10	U	C6ZE81	ACF98531	Δ9 desaturase-like protein	d9	Tree: close to 200 cyano and plant d9 and d7 desaturases	O		Looks like A8JEN2	
11	U	A8IQB8	XP_001691597.1	Plastid acyl-ACP desaturase	d9	Tree: close to 280 plant d9-ACP desaturases	C		FAB2, plastid acyl-ACP desa	
12	K	(A8HMA4) I2CYZ4	AFJ74144 XP_001690117	d4-Fatty acid desaturase	d4	Tree: close to d5 and other desaturases from several organisms	C	Contains CytB5	Plastidic, contains CytC, CrΔ4FAD	[46]

13	U	A8J2E8	XP_001695380	ω6 fatty acid desaturase-like protein	ω6 d12	Tree: close to 200 plant omega 6 desaturases	O		FAD6	
14	U	A8JEP6	XP_001701254	Fatty acid desaturase-like protein	d7	Tree: close to 200 cyano and plant d9 and d7 desaturases	C		FAD5b = d7	
15	U	A8IRD7	XP_001691564	Predicted protein, fatty acid desaturase	?	Tree: close to several fungal and other algal desaturases	SP		Many algae and plants have the same hypothetical protein.	
16	U	A8J3G2***	XP_001695919	Low-CO2 induced protein	d3t***	Tree: close to several plant hypothetical enzymes	O		d3-trans desaturase	
-		-		-	d11				d11-desaturase	

Table S3 – Fatty acid desaturases of *C. reinhardtii*. Some of these desaturases have been studied in vitro but many are putatively assigned using psi-blasting A. thaliana desaturases and phylogeny analysis. *) Known (K) or unknown (U) specificity of desaturases based on literature search (see references). **) Predalgo {Tardif, 2012 #154} prediction: PredAlgo computes a score for the three cellular compartments: the mitochondrion (M), the chloroplast (C), and the secretory pathway (SP) (Cscore, Mscore, and SPscore, respectively). When the three scores were below a certain cutoff, the protein was assigned to the "Other" (O) category ***) palmitate desaturase introduces a d3t unsaturation in palmitate and is encoded by

FAD4, AT4G27030, in *Arabidopsis thaliana*. Psi-Blasting this gene against *C. reinhardtii*, gives a good hit (43% identity) which is annotated as low-CO2-induced protein (A8J3G2) in *C. reinhardtii*.

Table S4 - The desaturases of *Thalassiosira pseudonana*

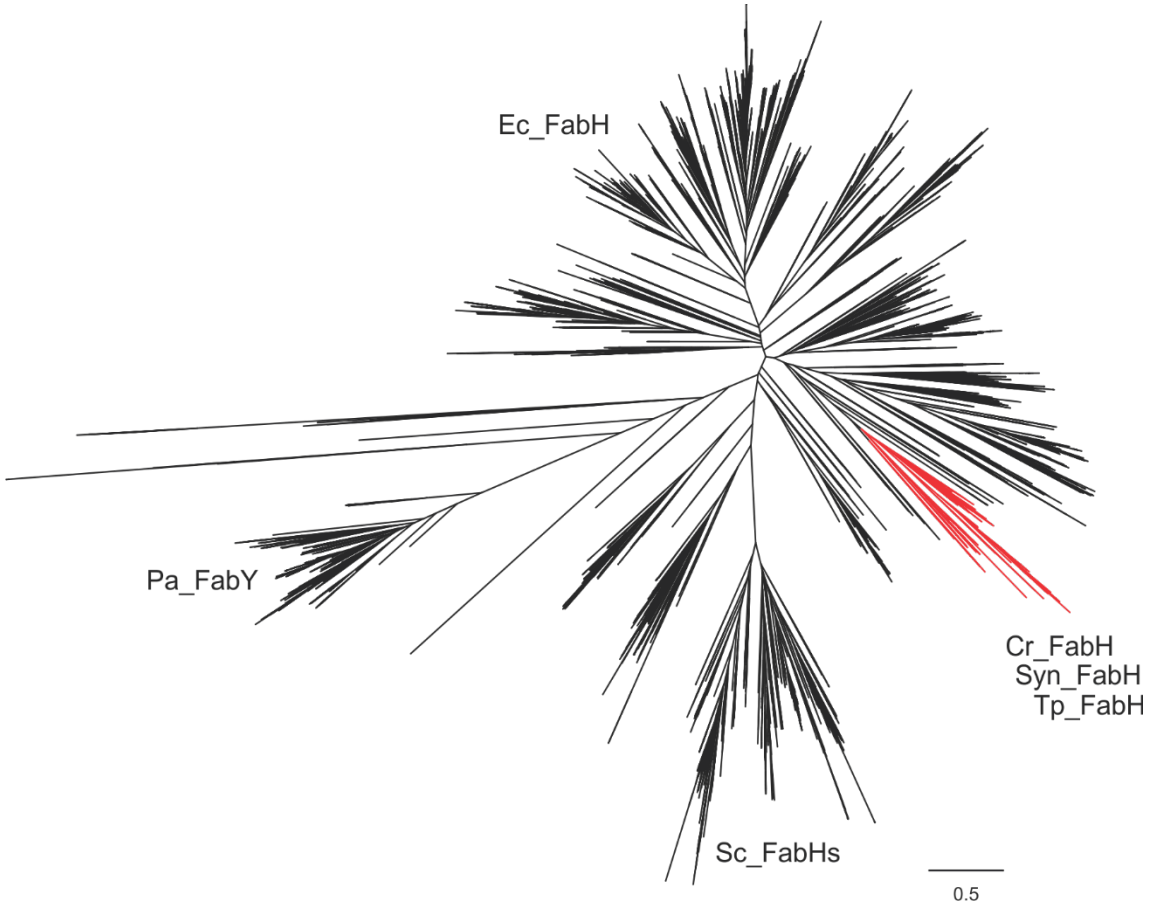
	Accession (NCBI)	Annotated protein name	Redox partner	Comments / FAD	Reference(s)
1	AAX14503.1	sphingolipid d8 desaturase with preference for dihydroxylated substrates	cytB5	**TpDesA**, d6 FADS like, Incubation with PUFA precursors after expression transgenic yeast yield no new FA peaks, prob not involved in PUFA biosynthesis	[47]
2	AAX14504.1	sphingolipid d8 desaturase with preference for dihydroxylated substrates	cytB5	**TpDesB** (TpDesB and TpDesD are now TpDesB). D6 FADS like, Incubation with PUFA precursors after expression transgenic yeast yield no new FA peaks, prob not involved in PUFA biosynthesis	[47]
3	XP_002289468.1	d6 fatty acid desaturase/d8 sphingolipid desaturase	cytB5	**TpDesE** (no full length DNA reads); Chr 4, d6 FADS like, d6 and d8 desaturases in diatoms, Ectocarpus, Guillardia, Ostreococcous, Leishmania	
4	XP_002290058.1	D5 or d11?	cytB5	**TpDesG**, d6 FADS like, d5 and d11 desaturases in Thalassiosira species, hydrophobic regions but not predicted as TM, Tonon states it is possibly a pseudogene/not expressed but it is expressed in RNAseq data	[47]
5	XP_002291529	Microsomal d6 desaturase DES3*, d6 desaturase involved in PUFA biosynthesis	cytB5	**TpDesI**, d6 FADS like, Transgenic yeast show expressed DESI desaturates C16:1, C18:1, and exogenous fatty acids at the C6 position, clusters with PtDEL6 d6 desaturase	[47]
6	AAX14506.1	d4 desaturase involved in PUFA biosynthesis	cytB5	**TpDesK**, d6 FADS like, also known as DES4	

7	XP_002296867	D5	cytB5	**TpDesM**, also known was DES2, lowly expressed, d6 FADS like	
8	XP_002296094.1 AAS75335.1	16:0 specific d11 desaturase	cytB5	**TpDesN**, also known was DES11, d6 FADS like	[48]
9	AAX14502.1	d5 desaturase on C20 FAs, involved in PUFA biosynthesis	cytB5	**TpDesO**, clusters with previously characterized d4 and d5 desaturases, PtDEL5 d5 desaturase	
10	XP_002291233	?	-	**TpDesH**, d6 FADS like, nonspecific	
11	XP_002297062.1	?	-	**TpDesL**, d6 FADS like, partial, lowly expressed	
12	XP_002297364.1	D9-ACP	ferr	TpDesd9ACP, DES7	
13	XP_002294497.1	d4 sphingolipid FADS like, nonspecific	-	hydrophobic regions but not predicted as TM	
14	XP_002291057.1	d12 FADS like, nonspecific	-	omega3 desaturas in Fistulifera, desaturase hits in algae, stearoyl-CoA d6 desaturase in C. elegans d15 phospholipid desaturase in Oscillatoria	

15	XP_002292071.1	d12 desaturase	-		
16	XP_002290033.1	stearoyl-CoA d9 desaturase			
17	XP_002286531.1	stearoyl-CoA d9 desaturase			
18	XP_002288176.1	D12		DES9, d12 FADS like	
19	XP_002287309.1	acyl desaturase/hydroxylase, sphingolipid d4 desaturase		d4 sphingolipid FADS like	
20		d9 FAS like		D9 acyl CoA	
21		d9 FAS like		D9 acyl CoA	

Table S4 – The desaturases of *T. pseudonana*. Many of these desaturases have been previously annotated by Tonon et al. [47] and these are labeled in bold as TpDesX. The other candidates are putative enzymes.

Figure S1 – Phylogeny of ketoacyl synthase III



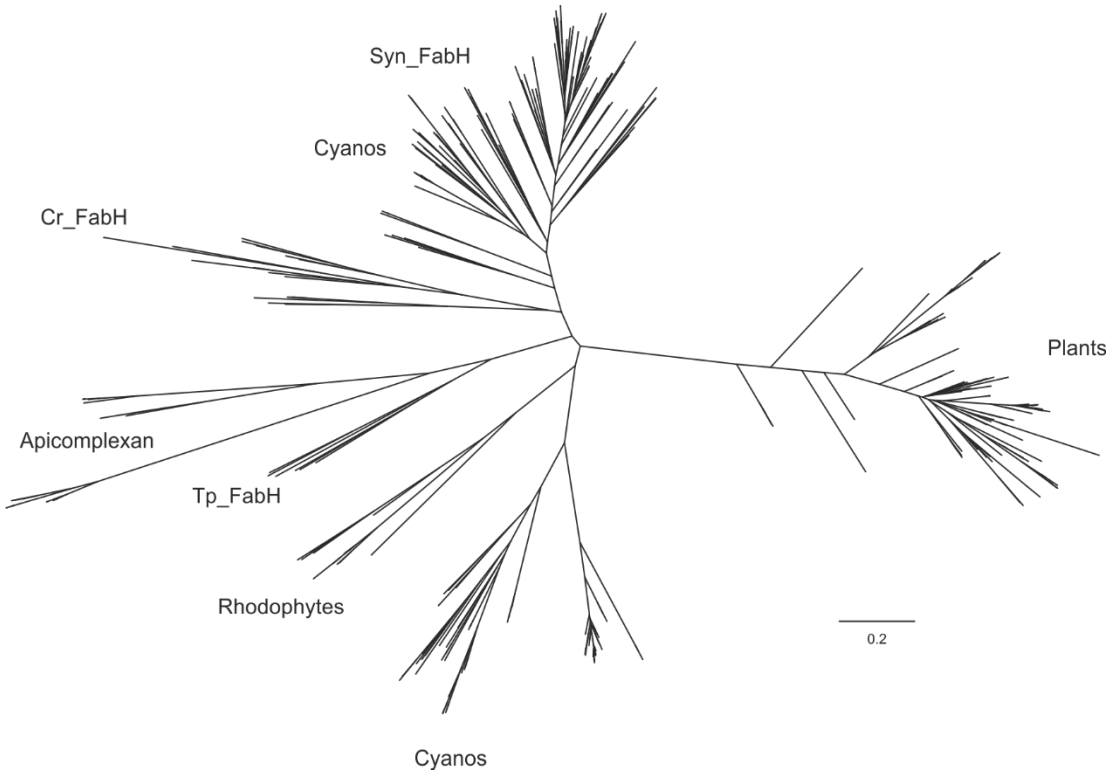


Figure S1 – Phylogeny of ketoacyl synthase III. *Top*: phylogenetic tree of >6000 KSIII/FabH sequences obtained by psi-blasting *E. coli*, *Synechocystis* sp PCC6803, *C. reinhardtii* and *T. pseudonana* KSIII/FabHs against the complete NCBI database, addition of *Pseudomonas* FabY, followed by removal of duplicates, alignment using Muscle[49] and tree construction using Fasttree.[50] The red labeled clade contains Tp, Cr and Syn FabHs. *Bottom*: extracted tree showing the phylogenetic relatedness of plant, algal and cyanobacterial FabHs. It should be noted that only one FabH per cyanobacterial, algal or plant species was detected, in contrast to some bacteria.

Figure S2 - Phylogeny of acyl-acyl carrier protein synthetases (AasSs)

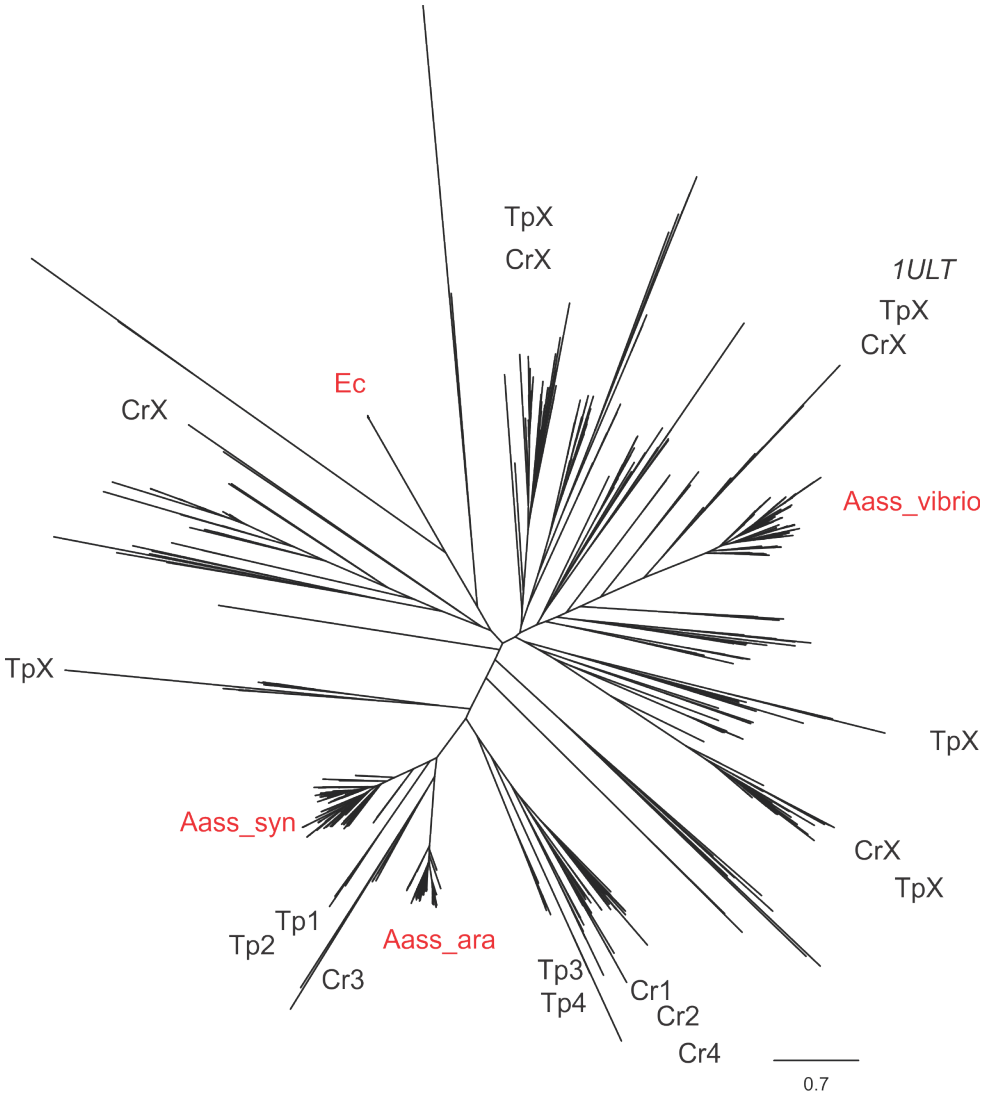


Figure S2 – Phylogeny of acyl-acyl carrier protein synthetases (AasSs). Four AasSs have been characterized in the literature, highlighted in red (Ec is the *E. coli* unusual membrane associated bifunctional AasS, Aass_syn is Slr1609 from *Synechocystis* sp PCC 6803, Aass_ara is AAE15 from *Arabidopsis thaliana* and Aass_vibrio is AasS from *V. harveyii*. With these as seeds, we assembled a sequence alignment of putative enzymes in bacteria, algae and cyanobacteria. By directly blasting the known AasSs against *T. pseudonana* and *C. reinhardtii* we identified the four best candidates in both organisms (labeled Tp1-4 and Cr1-4) whereas the proteins labeled with CrX or TpX were only found by assembling this large (~1000 sequences) sequence alignment and tree.

Table S5 - GCMS analysis of *E. coli* cultures in M9 fed with various fatty acids.

Shown are percentages of total fatty acids, determined by GCMS of fatty acid methyl esters. Data of feeding C11, C12, C13, C14, C16 and C18 is not shown.

	C14:0		C15:0		C16:0		C16:1d9		C17:0		CyC17		C18:0		CyC19		C18:1	
C1	2.4	0.2	0.0	0.0	56.8	4.1	7.9	1.3	0.0	0.0	13.0	1.5	4.8	0.3	14.5	1.8	0.6	0.1
C2	2.5	0.3	0.0	0.0	63.3	3.3	4.1	0.0	0.0	0.0	12.1	1.8	5.8	0.2	11.0	1.3	1.1	0.4
C3	1.9	0.0	6.1	1.0	53.2	2.7	3.9	0.9	3.8	2.7	14.2	0.3	5.8	1.2	9.3	2.3	1.7	0.2
C4	2.1	0.6	0.0	0.0	64.0	2.7	3.1	0.3	0.0	0.0	12.4	1.4	6.2	0.0	9.8	1.3	2.4	0.9
C5	3.2	0.6	0.8	1.2	61.9	1.0	2.7	0.7	0.2	0.0	15.6	1.6	7.5	0.4	3.9	0.5	4.2	1.0
C6	4.1	0.1	0.0	0.0	67.2	2.0	3.2	1.1	0.0	0.0	11.8	2.0	8.4	1.3	3.4	1.5	1.9	1.3
C7	2.9	0.2	0.0	0.0	60.9	5.3	4.7	0.1	0.0	0.0	15.0	2.5	5.8	1.1	9.4	1.3	1.4	0.6
C8	3.2	0.5	0.0	0.0	62.3	3.9	6.6	0.8	0.0	0.0	11.8	0.1	4.8	1.0	11.1	1.3	0.3	0.4
C9	2.4	0.5	0.0	0.0	61.0	1.2	8.4	0.2	0.0	0.0	10.2	1.2	4.1	0.0	13.9	1.6	0.0	0.0
C10	6.9	2.8	0.0	0.0	68.9	3.1	6.9	0.2	0.0	0.0	6.7	9.5	9.1	7.5	1.5	2.1	0.0	0.0
control	2.3	0.2	0.2	0.3	57.3	0.6	8.7	0.1	0.0	0.0	13.8	0.3	3.8	0.1	13.0	0.8	1.0	0.1

Table S5 - GCMS analysis of *E. coli* cultures in M9 fed with various fatty acids. Cultures were grown for 4h at 37 °C and supplemented with various fatty acids and grown o/n at room temperature. The cultures were centrifuged, washed, and FAMEs made directly from the cell pellet prior to hexane extraction and GCMS analysis.

Table S6 - Feeding fatty acids to *Synechocystis* sp PCC 6803

	heptadecane	heptadecene	C14:0	C15:0	C16:0	C16:1	14meC17	C17:1	C17:2	C18:0	C18:1	C18:1	C18:2	C18:3	C18:3	C18:4
c1	13.2	12.2	0.9	0.0	38.2	5.8	0.3	0.7	0.4	0.6	3.1	0.7	9.6	10.7	2.4	1.3
c2	9.3	10.2	1.1	0.0	38.9	6.7	0.5	0.7	0.5	0.6	2.7	0.6	9.3	14.0	3.0	1.9
c3	11.2	11.0	1.0	0.0	41.6	0.6	1.3	0.7	0.6	3.2	0.7	0.3	9.5	13.6	2.9	1.9
c4	10.1	10.1	1.0	0.0	38.4	6.6	0.4	0.8	0.5	0.5	2.8	0.6	9.6	14.1	2.8	1.7
c5	11.8	13.0	0.9	0.0	34.9	7.0	0.6	1.1	0.6	0.4	2.5	0.6	8.3	13.7	2.8	1.8
c6	11.5	0.5	1.2	0.0	41.7	7.4	0.6	0.7	0.5	0.6	3.0	0.7	10.6	15.6	3.3	2.0
c7	14.0	19.2	1.5	0.0	33.7	5.7	0.0	1.0	0.0	0.0	2.4	0.0	6.9	11.1	2.7	1.8
c8	10.6	13.0	1.7	0.0	37.1	7.2	0.0	0.7	0.4	0.5	2.3	0.6	8.2	13.2	2.8	1.8
c9	5.8	8.6	1.1	1.3	41.0	5.9	1.6	3.4	2.1	0.5	3.3	0.7	7.4	12.6	3.0	1.7
c10	5.2	5.8	0.8	0.0	45.6	7.2	0.4	0.7	0.5	0.6	2.4	0.9	9.4	15.5	3.2	1.8
c11	2.6	53.7	1.0	3.8	16.8	2.4	1.8	5.3	2.5	0.5	0.4	0.3	2.5	4.6	1.2	0.6
c12	6.8	11.0	11.2	0.0	40.8	8.9	0.0	0.0	0.0	1.4	0.0	0.0	6.4	9.5	2.4	1.6
c13	2.8	7.2	2.5	14.2	30.9	3.4	5.8	11.9	4.5	1.3	1.9	0.5	3.8	6.2	2.5	0.7
c14	0.8	1.0	90.4	0.0	4.4	0.7	0.1	0.1	0.1	0.2	0.1	0.0	0.7	1.0	0.3	0.1
c16	1.3	1.3	0.7	0.1	89.2	1.2	0.2	0.1	0.1	0.5	0.5	0.2	1.9	2.2	0.5	0.2
c18	1.9	2.0	0.2	0.0	2.5	0.2	0.1	0.3	0.1	91.4	0.3	0.1	0.5	0.4	0.1	0.0
control	9.2	10.2	0.9	0.0	37.7	6.2	2.9	0.6	0.5	0.6	2.6	0.7	9.1	13.7	3.2	1.9

Table S6 – Feeding fatty acids to *Synechocystis* sp PCC 6803. Shown are percentages of total fatty acids, determined by GCMS.

Figure S3 – Feeding acids to *E. coli* overexpressing VhAasS

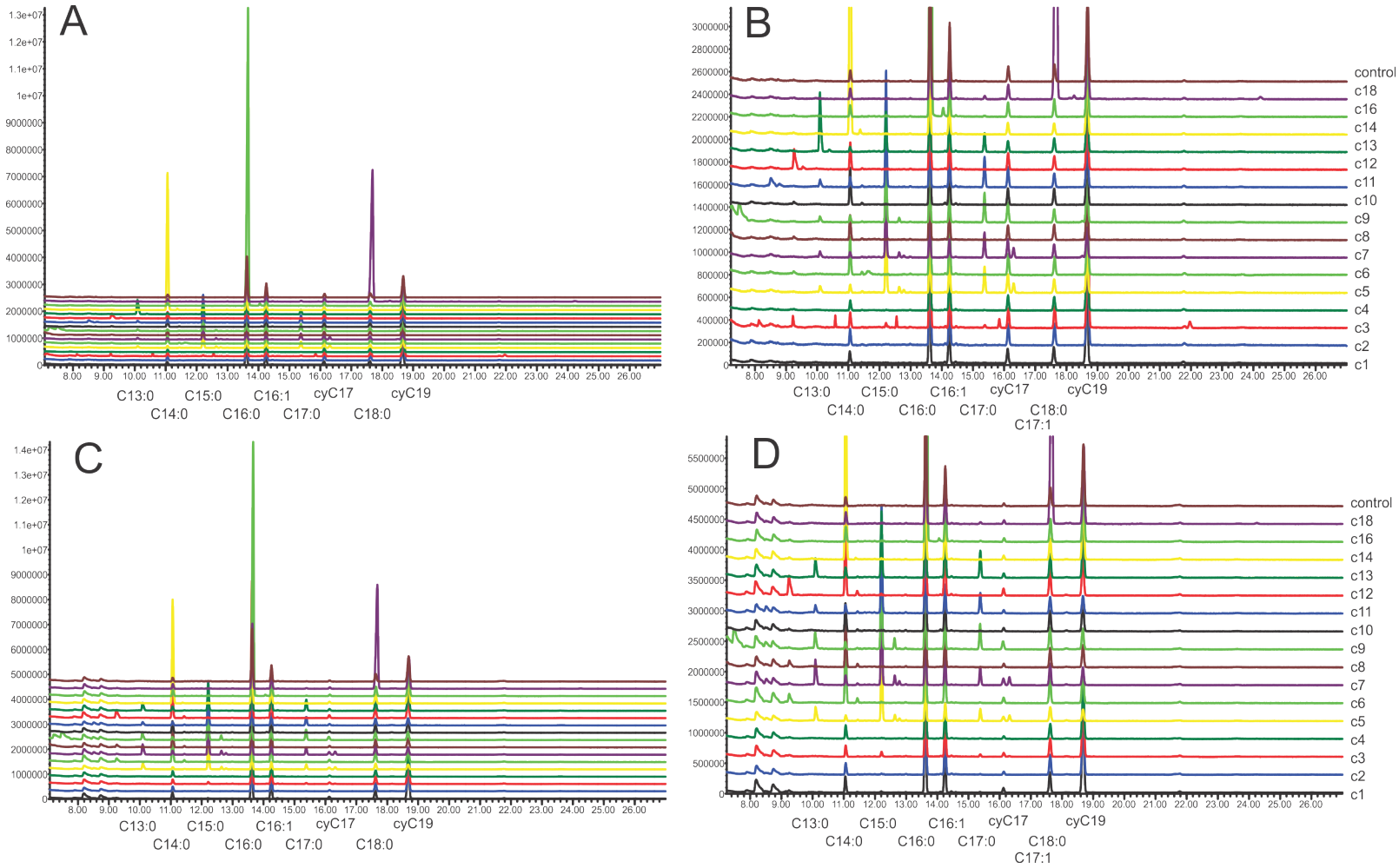


Figure S3 - Feeding acids to *E. coli* overexpressing VhAasS. GCMS chromatograms of FAMEs made directly from fed and washed *E. coli* cultures. The y-axis represents arbitrary abundance and the x-axis is time (in minutes). For this experiment, *E. coli* BL21 was transformed with pSU20VhAasS and this strain was fed with 1 mM fatty acids C1-C18 from the beginning of the experiment (A,B) or at an OD of 0.8 (C,D). Panels B and D are zoomed-in version of A and C. The labels on the X-axis are the observed fatty acids by GCMS and the chromatograms are labeled on

the traces themselves on the right. Only in the samples supplemented with C3, C5, C7, C9, C11, C13 new peaks are observed that correspond with the fatty acids C13, C15, C17, C17:1.

Figure S4 – Feeding acids to Slr1609 knock out *Synechocystis* strain

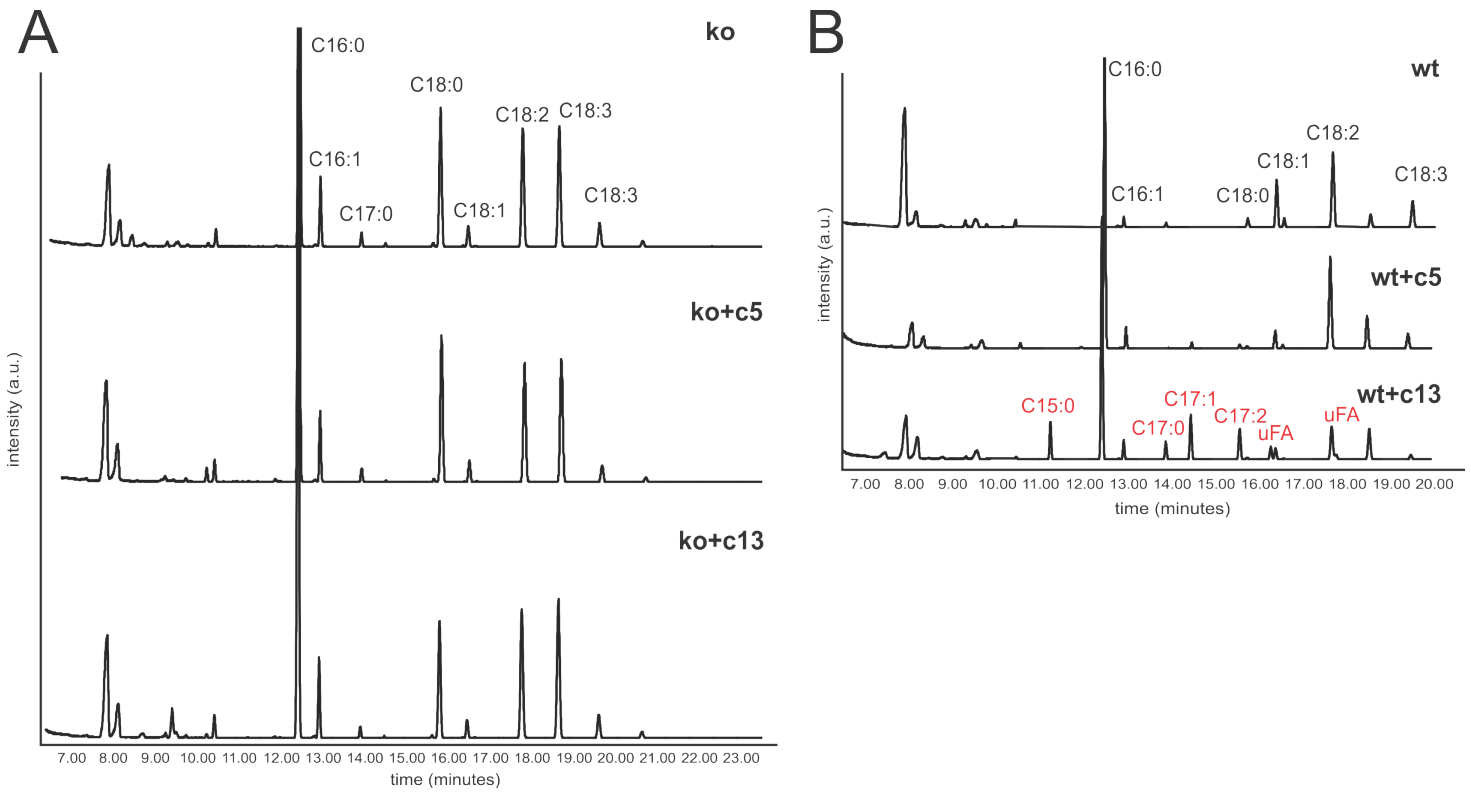


Figure S4 - Feeding acids to Slr1609 knock out *Synechocystis* strain. Representative FAME GCMS chromatograms of feeding acids to *Synechocystis* strains. A) the Slr1609 knock-out strain was supplemented with C5 (valeric acid) or C13 (tridecanoic acid). The top chromatogram is a control. B) *Synechocystis* sp PCC 6803 was supplied with C5 or C13. The top chromatogram is a control.

Figure S5A – Feeding fatty acids to *Chlamydomonas reinhardtii* (feeding exponential phase culture)

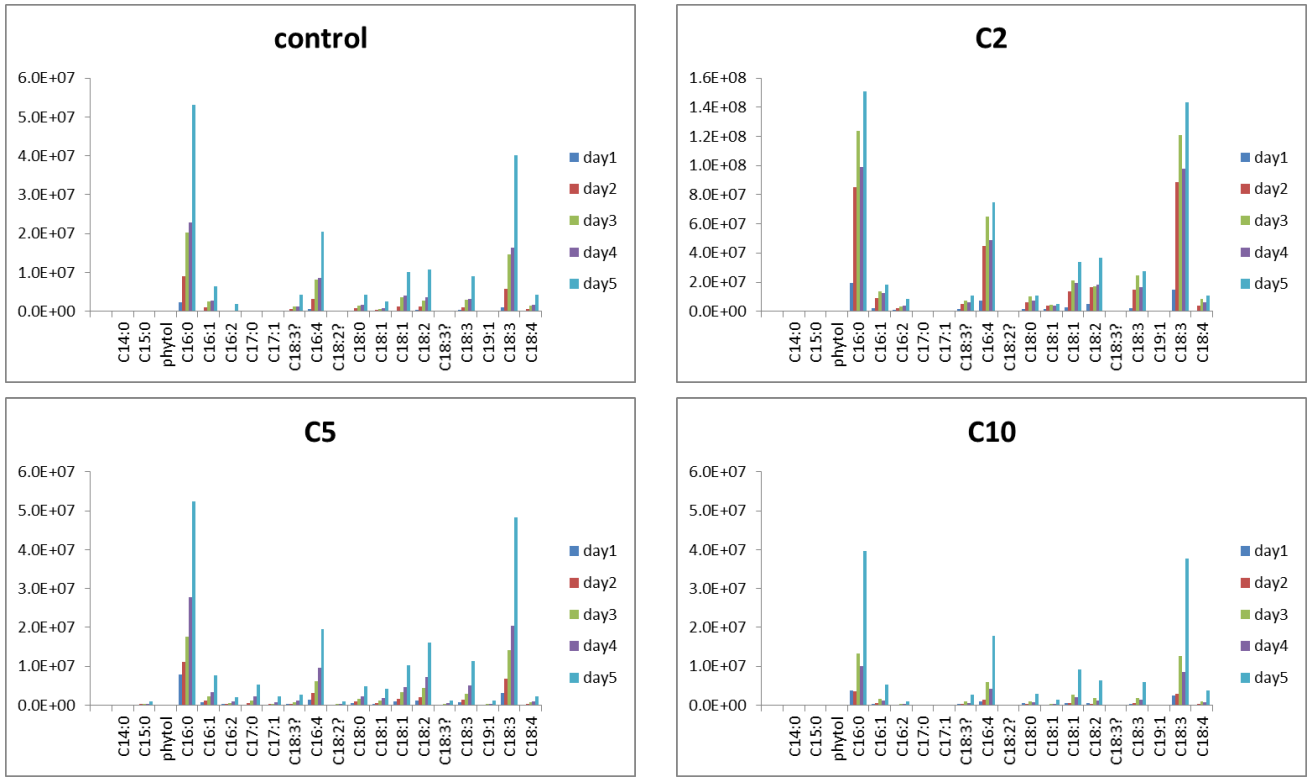


Figure S5B – Feeding fatty acids to *Chlamydomonas reinhardtii* (feeding exponential phase culture, zoom in)

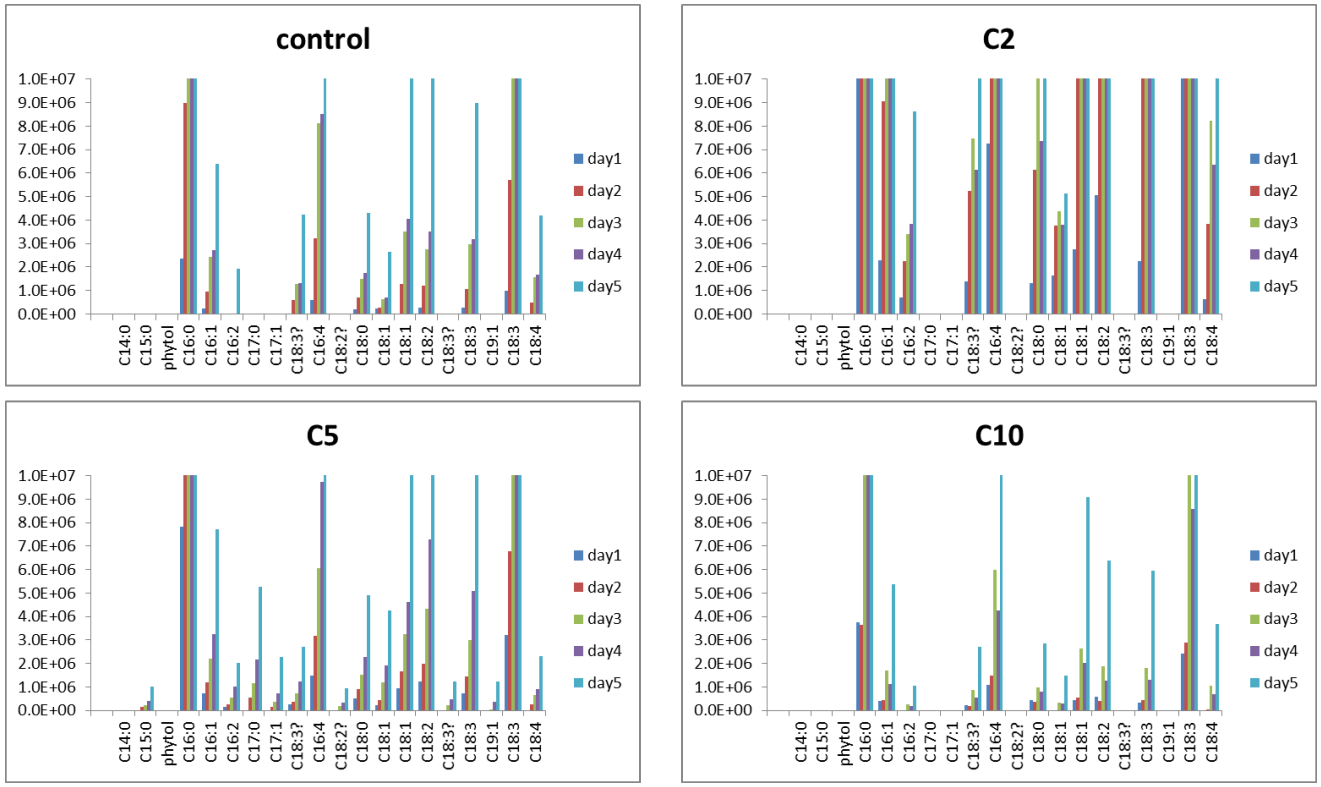


Figure S5C – Feeding fatty acids to *Chlamydomonas reinhardtii* (feeding stationary phase culture)

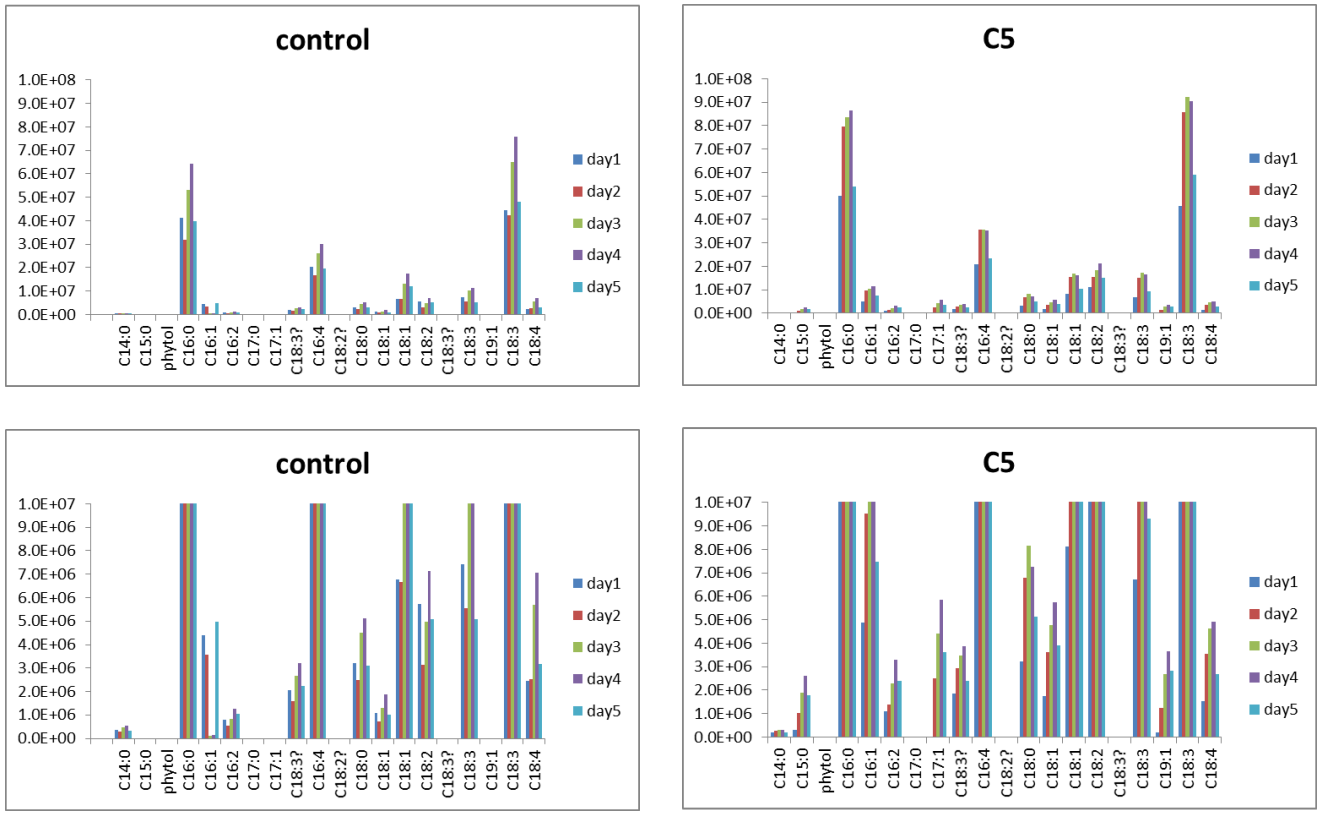


Figure S5 – Feeding fatty acids to *Chlamydomonas reinhardtii*. A) feeding C2, C5, C10 and control from the start of a very dilute culture for 6 days, B) zoom-in on small peaks in A; C) feeding C5 and control to dense culture. Cultures were spun down and FAMEs made directly from biomass and analyzed by GCMS.

Table S7 - Feeding fatty acids to *Thalassiosira pseudonana*

	CNTRL	C2	C3	C4	C5	C6	C7	C8	C9	C10
C12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.98	0.00	0.00
C14:0	15.55	17.22	18.95	21.85	23.44	25.58	23.72	28.87	19.07	36.06
C15:0	0.56	0.00	13.87	0.00	1.47	0.00	0.87	0.00	0.00	0.00
C16:0	30.47	39.32	32.36	47.92	33.63	36.22	30.94	28.58	36.72	26.25
C16:1[1]	5.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16:1	25.52	25.43	15.94	18.90	22.24	15.87	16.63	6.80	13.62	11.29
C16:1[2]	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16:2[3]	0.53	1.72	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00
Cx:2[4]	2.16	0.00	1.17	0.00	0.55	0.00	1.08	0.00	0.00	0.00
C16:3	7.26	5.90	3.89	3.07	4.14	6.49	7.49	3.26	8.08	8.04
C18:0	0.00	0.00	0.00	0.00	1.18	4.26	7.38	2.95	4.29	0.00
C18:1	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00
C18:4[5]	4.10	4.02	3.96	0.98	4.61	0.00	1.02	0.76	0.00	0.00
cx:1[6]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.14	0.00
C20:5	7.73	6.20	8.28	5.41	7.68	11.58	9.27	5.80	13.08	18.36
C22:6[7]	0.26	0.00	1.58	1.40	0.64	0.00	1.60	0.00	0.00	0.00

Table S7 - Feeding fatty acids to *Thalassiosira pseudonana*. Various neutralized fatty acids were fed to the diatom and fatty acid profiles analyzed by GCMS. [1,2,3,5,7] are tentatively assigned. [4] is most likely another C16:2 isomer. [6] is only observed in the sample fed with nonanoic acid and shows a MS pattern of an unsaturated fatty acid, not matching to any hit in the NIST database.

Figure S6 - Feeding fatty acids to *Thalassiosira pseudonana*

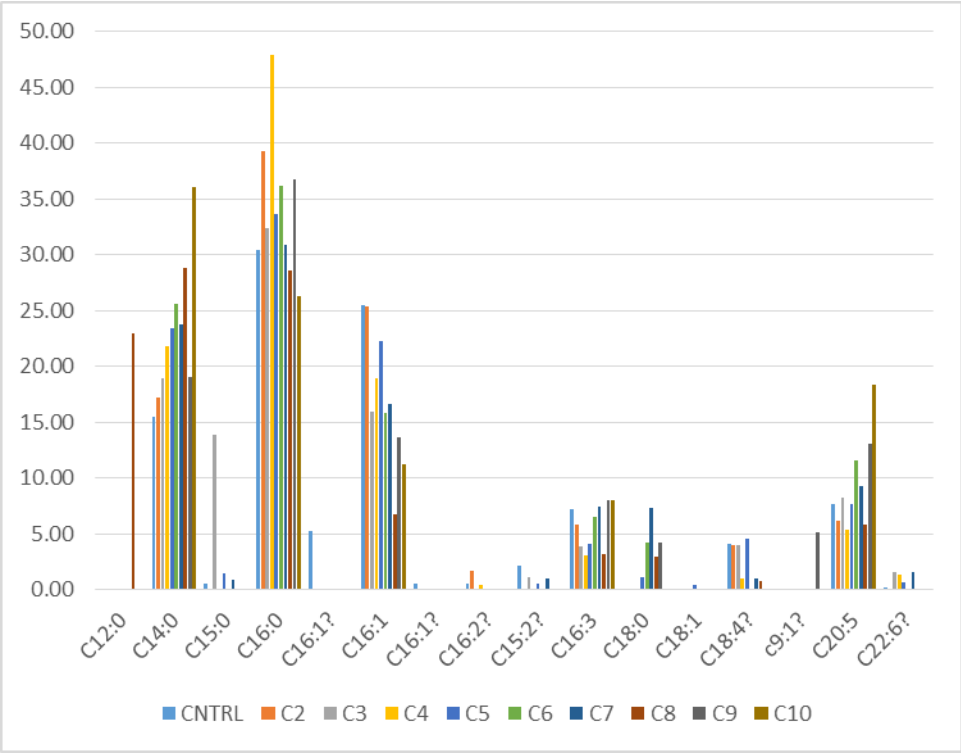
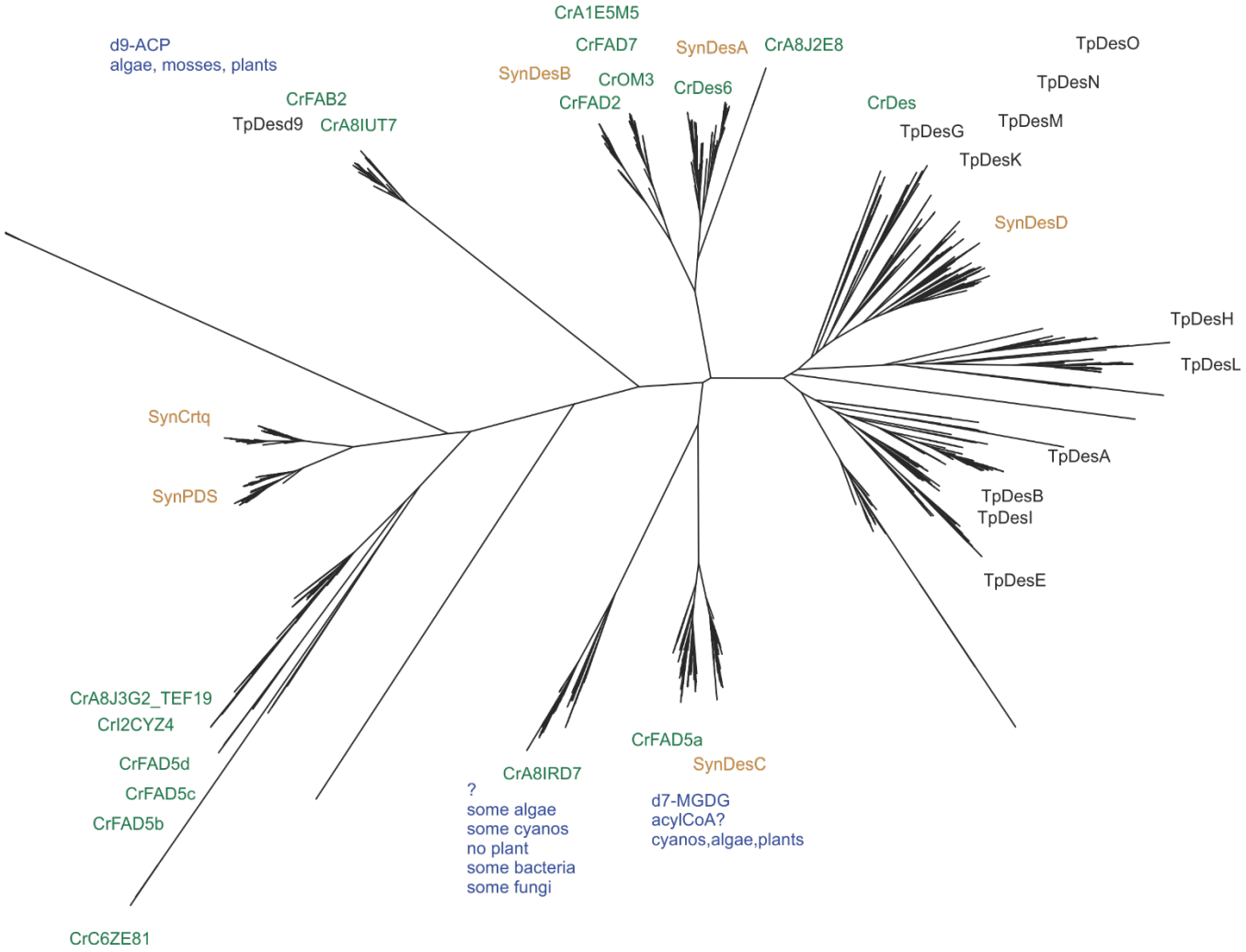


Figure S6 - Feeding fatty acids to *Thalassiosira pseudonana*. Various neutralized fatty acids were fed to the diatom and fatty acid profiles analyzed by GCMS. Graphical representation of the data in Table S7.

Figure S7 - Phylogeny of desaturases including diatoms

C. reinhardtii = green
T. pseudonana = black
Synechocystis 6803 = orange



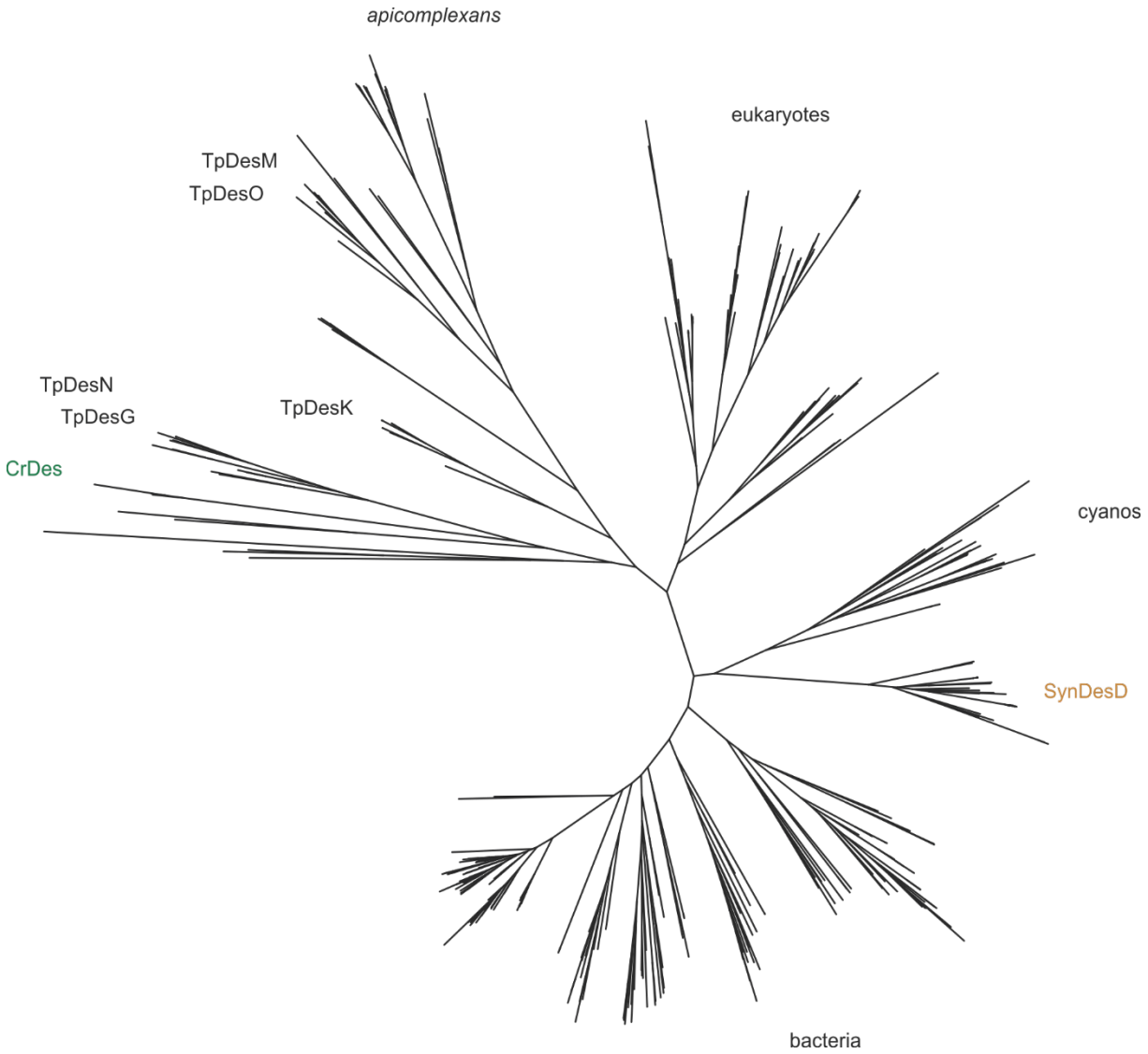

apicomplexans
eukaryotes
TpDesM
TpDesO
TpDesN
TpDesG
TpDesK
CrDes
cyanos
SynDesD
bacteria

Figure S7 – Phylogeny of desaturases including diatoms. *Top*: a phylogenetic tree was constructed from unique sequences of desaturases of *Synechocystis* sp PCC 6803 (brown), *C. reinhardtii* (green) and *T. pseudonana* (black). The ~1000 sequences were aligned using Muscle and the tree constructed using Fasttree. *Bottom*: extracted tree visualizing the relatedness of CrDes and TpDesN/G, and to a lesser extent SynDesD.

Table S8 - Feeding desaturase inhibitors to *Synechocystis* sp PCC 6803

	C14:0		C16:0		C16:1d9		C18:0		C18:1d9		C18:2d9,12		C18:3d6,9,12		ufa/sfa	
	av	sd	av	sd	av	sd	av	sd	av	sd	av	sd	av	sd	av	sd
wt	4.63	0.54	62.28	0.38	6.47	0.45	1.82	0.21	1.56	0.10	12.37	0.22	10.86	0.07	0.45	0.00
S9	6.05	1.87	72.06	0.34	2.37	0.25	2.84	0.32	7.12	1.52	5.54	1.51	4.03	0.97	0.24	0.02
S10	11.37	2.99	66.93	0.59	7.43	0.87	2.61	1.19	0.50	0.71	4.36	2.07	6.80	2.85	0.24	0.07
S11	9.08	1.57	66.95	0.22	4.45	0.28	6.20	0.84	1.01	0.13	5.70	0.05	6.61	0.31	0.22	0.01

Table S8 - Feeding desaturase inhibitors to *Synechocystis* sp PCC 6803. Shown are percentages of total fatty acids, determined by GCMS.

Figure S8 – Feeding desaturase inhibitors to *Synechocystis* sp PCC 6803

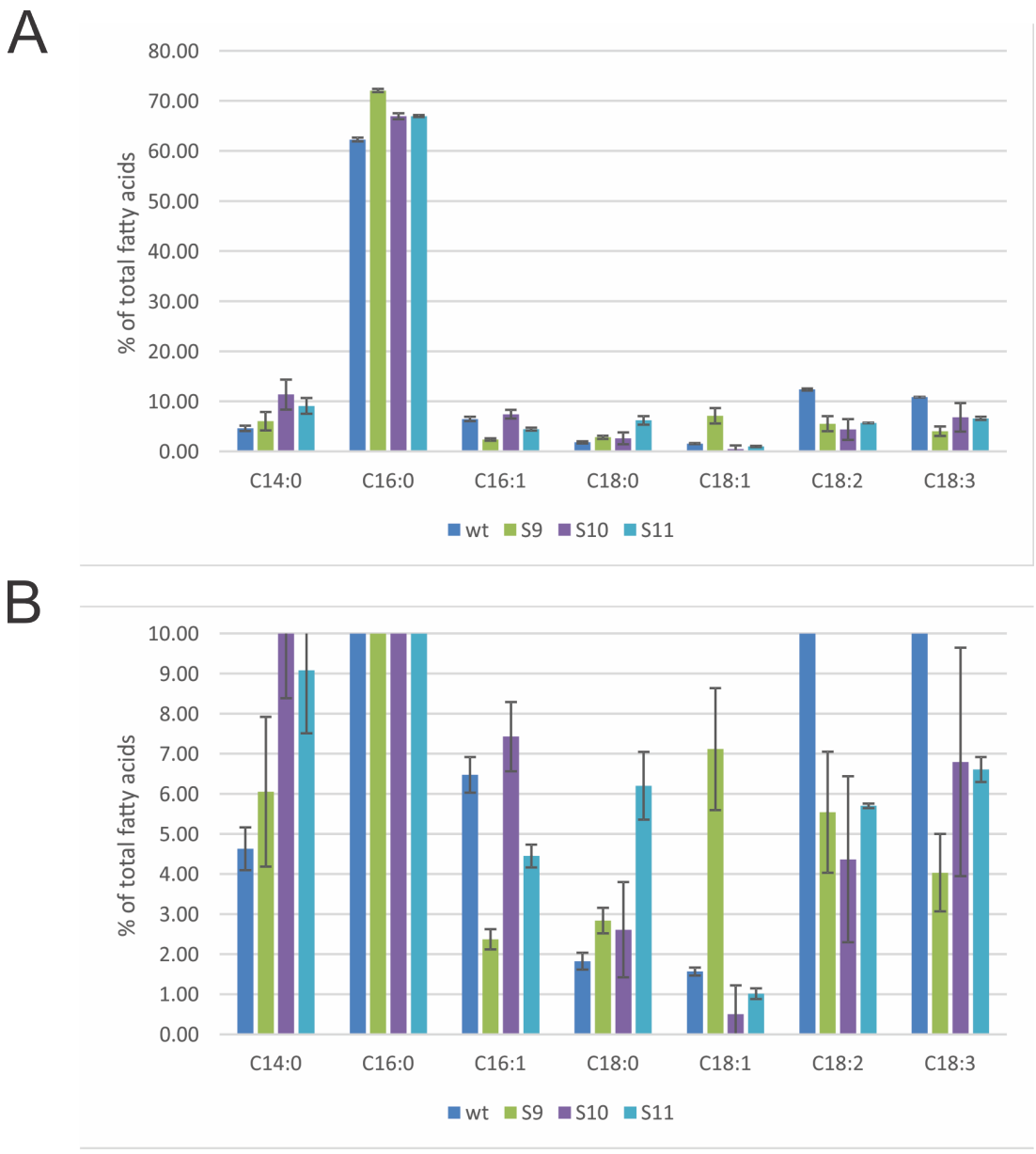


Figure S8 - Feeding desaturase inhibitors to *Synechocystis* sp PCC 6803. A) overview of fatty acid profile of cyanobacterium *Synechocystis* sp PCC6803 fed with various thia fatty acids and B) zoom-in on less abundant fatty acids. Graphical representation of Table S8.

Table S9 - Fatty acid degradation across species

				E. coli	*Synechocystis 6803*	*cyanobacteria*	*C. reinhardtii*	[53]	*T. pseudonana*
		FadL	transporter	NP_416846.2	no hits	no hits	no hits		no hits
		FadD	acyl-CoA synthetase	NP_416319.1	WP_010873726	many hits	XP_001700210.1		XP_002289936.1
					WP_041428167		XP_001700230.1		six more
					WP_010873549		XP_001702947.1		
							XP_001702039.1		
		FadE	acyl-CoA dehydrogenase	NP_414756.2	no hits	many hits but none in *Synechocystis*	no hits	ech1 (dual)	XP_002296360.1
								ech2	
		FadB	enoyl-CoA hydratase	NP_418288.1	no hits	only hits in mastigocladus and scytonema	XP_001699366.1	ech1 (dual)	XP_002292674.1
								dci1	
		FadA	acetyl-CoA acyltransferase	YP_026272.1	WP_041425968	many hits	XP_001697225.1	ato1	XP_002296579.1
							XP_001694888.1		XP_002291557.1
									XP_002288423.1
									XP_002291097.1
	FadB, YfcX	FadJ	3-hydroxyacyl-CoA dehydrogenase	NP_416843.1	no hits	only hits in mastigocladus and scytonema	XP_001699366.1		XP_002292674.1
									XP_002287474.1
	FadA, YfcY	FadI	3-ketoacyl-CoA thiolase	NP_416844.1	WP_041425968	many hits	XP_001697225.1		XP_002288423.1
							XP_001694888.1		XP_002291557.1
									XP_002296579.1
									XP_002291097.1

		FadR	regulator	NP_415705.1	no hits	no hits	no hits		no hits
FadK	FadD	YdiD	acyl-CoA synthetase	NP_416216.4	WP_010873726	many hits	XP_001700210.1		XP_002289936.1
							XP_001702039.1		XP_002287843.1
							XP_001700230.1		XP_002289865.1
									XP_002291517.1
									XP_002294268.1
									XP_002290752.1
breakdown unsatured acids			Peroxisomal 2,4-dienoyl-CoA reductase	no hits (KRs)	WP_041428273	many hits	XP_001701501.1		XP_002291829.1
			acyl-CoA oxidases	no hits	no hits	many hits	four		

Table S9 – Fatty acid degradation across species. Fatty acid degradation (beta-oxidation) enzymes are well known in E. coli. With these as seeds, we psi-blasted all enzymes against the genomes of *Synechocystis* sp PCC 6803, other cyanobacteria, *C. reinhardtii* and *T. pseudonana*, thereby populating the table with all putative enzymes found in these different organisms.

Figure S9 - Phylogeny of FadE

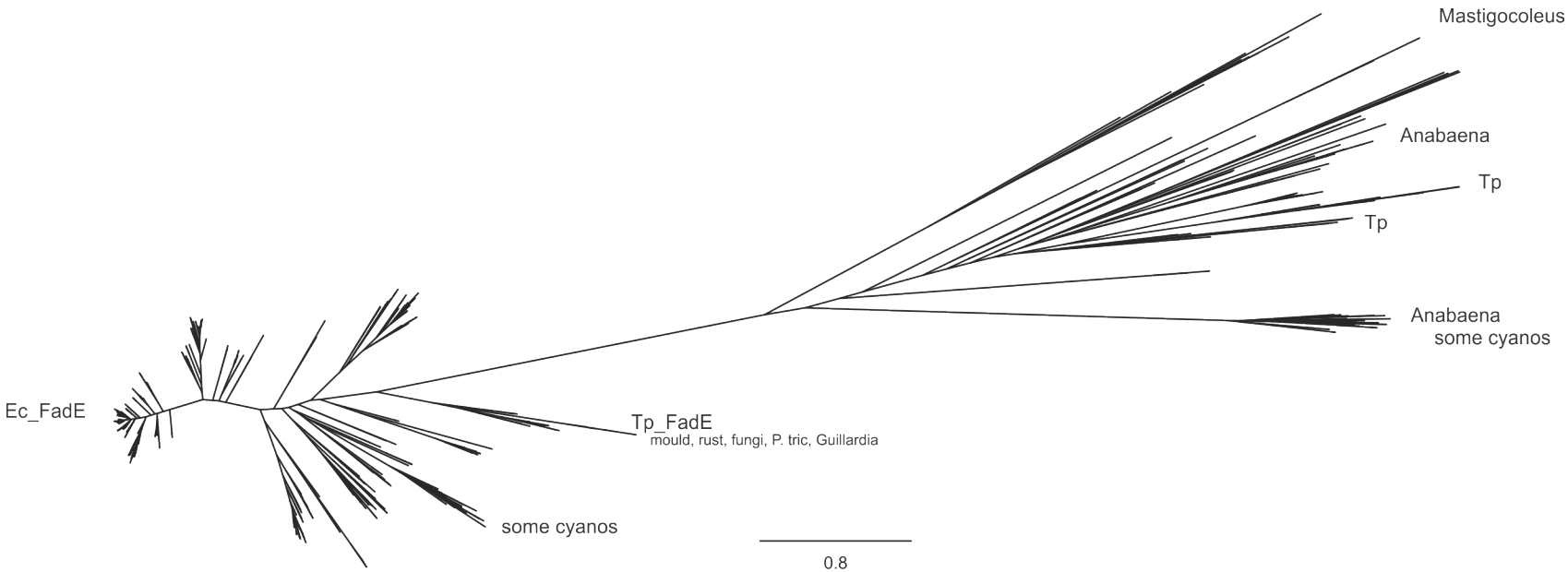


Figure S9 – Phylogeny of FadE. Using *E. coli* FadE and the putative hits in cyanobacteria and *T. pseudonana*, we constructed a phylogenetic tree of FadE proteins using Muscle and Fasttree. In *E. coli*, FadE is an acyl-CoA dehydrogenase and relatively close homologs can be found in some cyanobacteria, *T. pseudonana*, some moulds, rusts, fungi, *P. tricornutum* and *Guillardia*.

Figure S10 - Phylogeny of FadB/FadJ

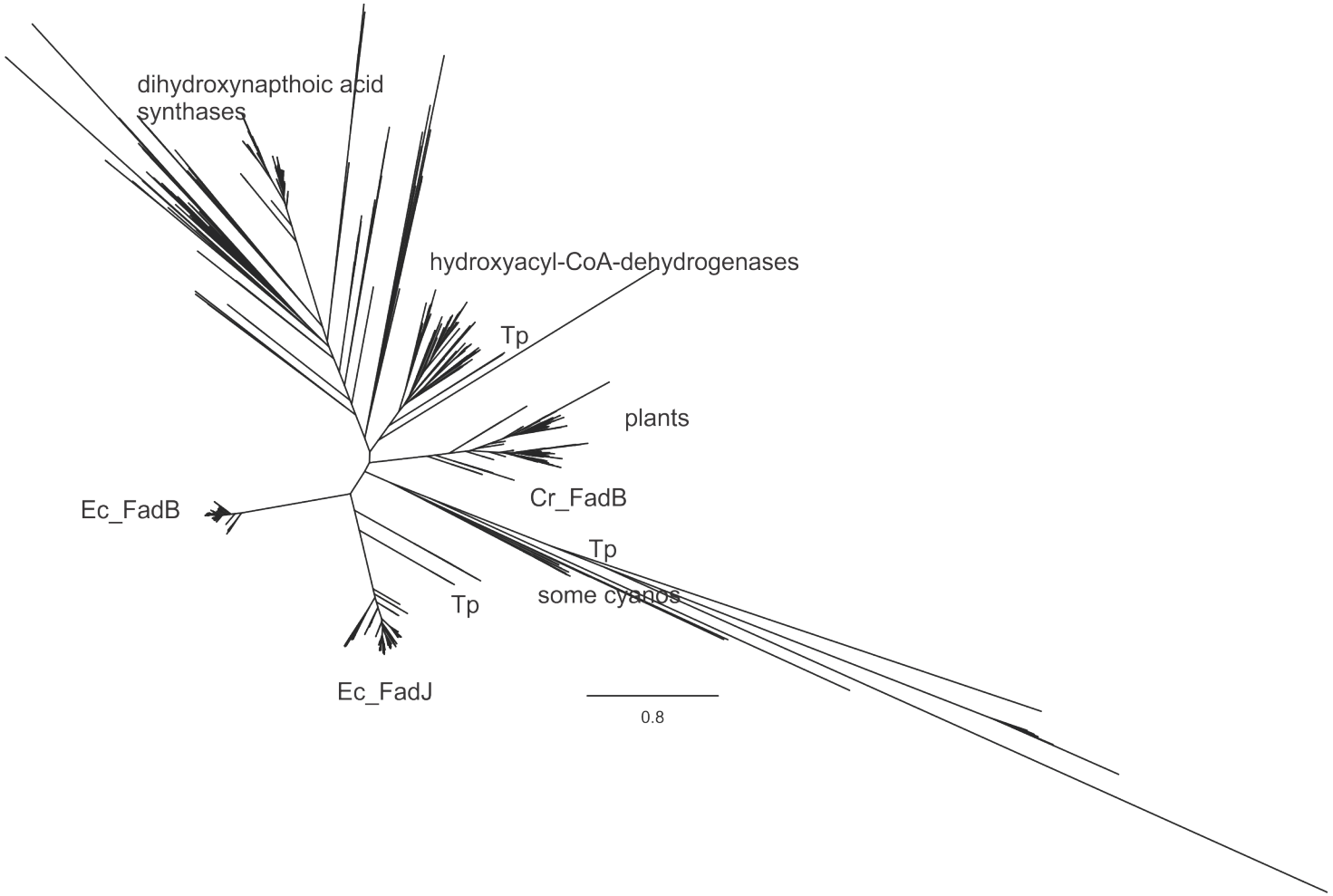


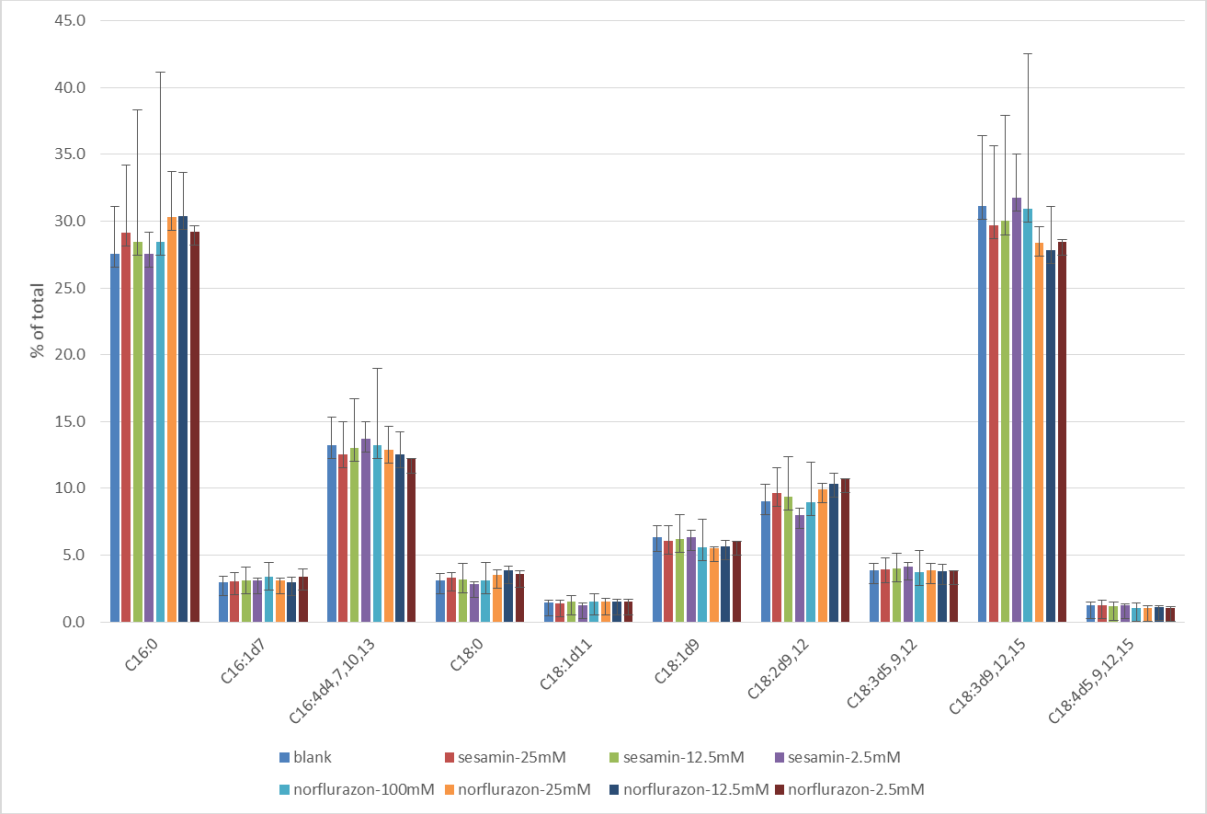
Figure S10 – Phylogeny of FadB/FadJ. Using *E. coli* Fadb/FadJ and the putative hits in cyanobacteria and algae, we constructed a phylogenetic tree of these proteins using Muscle and Fasttree. In *E. coli*, FadB and FadJ are the fused 3-hydroxyacyl-CoA dehydrogenase/ enoyl-CoA hydratase from either aerobic or anaerobic fatty acid degradation pathways. No candidate genes are observed in *Synechocystis* sp PCC 6803 but in some other cyanobacterial species. Tp are *T. pseudonana* hits.

Table S10 – Feeding thia fatty acids to *C. reinhardtii*

		control	S9	S10	S11
C16:0	av	31.1	28.7	35.1	34.3
0	sd	5.6	2.0	10.6	6.3
C16:1d7	av	4.4	4.9	5.3	4.7
	sd	1.6	1.1	3.0	2.8
C16:4d4,7,10,13	av	12.9	14.1	10.6	9.8
	sd	1.6	0.8	2.8	5.9
C18:0	av	4.5	2.4	7.3	5.4
	sd	3.8	0.2	6.6	4.7
C18:1d11	av	4.3	5.7	7.1	9.9
	sd	2.8	2.8	5.3	3.2
C18:1d9	av	4.7	5.1	4.9	4.2
	sd	0.8	0.5	1.0	1.2
C18:2d9,12	av	8.1	7.3	4.7	4.5
	sd	2.0	1.3	0.1	1.4
C18:3d5,9,12	av	3.7	4.1	2.8	3.0
	sd	0.3	0.4	1.0	0.6
C18:3d9,12,15	av	25.3	27.0	21.9	23.5
	sd	2.8	3.1	3.5	3.3
C18:4d5,9,12,15	av	0.9	0.8	0.5	0.7
	sd	0.5	0.2	0.7	0.6
ufa/sfa	av	1.9	2.2	1.6	1.6
	sd	0.7	0.2	1.0	0.7

Table S10 – Feeding thia fatty acids to *C. reinhardtii*. Shown are the relative percentages of fatty acids calculated based on the total FAMEs analyzed by GCMS. Thia fatty acids were fed to *C. reinhardtii* at 1 mM.

Figure S11 - Effect of norflurazon, sesamin or propylgallate on *C. reinhardtii*



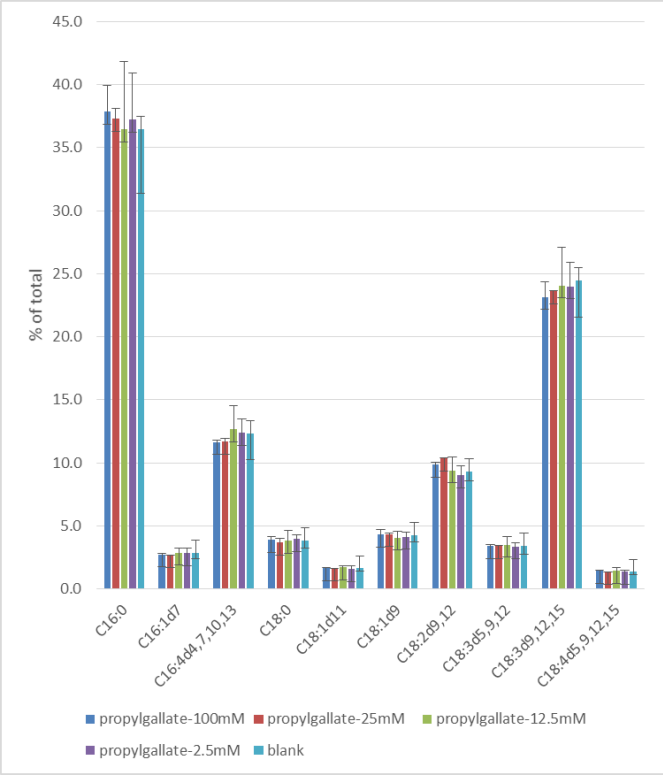


Figure S11 - Effect of norflurazon, sesamin or propylgallate on *C. reinhardtii*. Wildtype *C. reinhardtii* was fed with norflurazon (100 mM to 2.5 mM), sesamin (25 mM to 2.5 mM) or propylgallate (100 mM to 2.5 mM) in TAP media, and the fatty acid profile analyzed by GCMS after production of FAMEs and extraction with hexanes.

Figure S12 – Feeding thia fatty acids to *T. pseudonana*

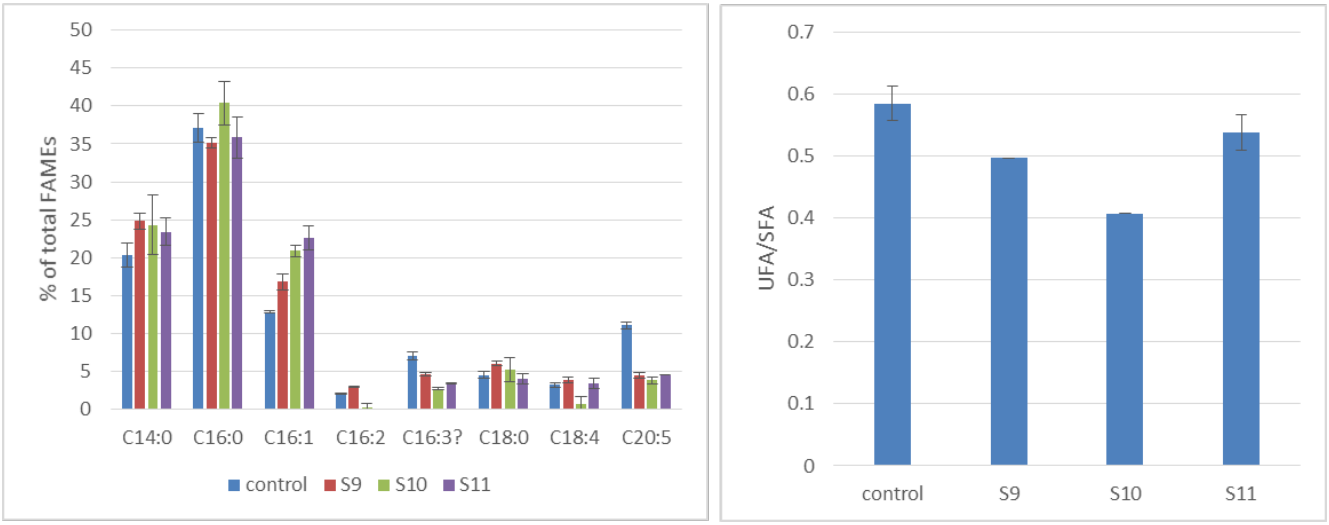


Figure S12 – Feeding thia fatty acids to *T. pseudonana*. Overview of effects on fatty acid profiles of thia fatty acids on *T. pseudonana*. UFA/SFA is the ratio of unsaturated fatty acids over saturated fatty acids.

Figure S13 - Phylogeny of *T. pseudonana* desaturases

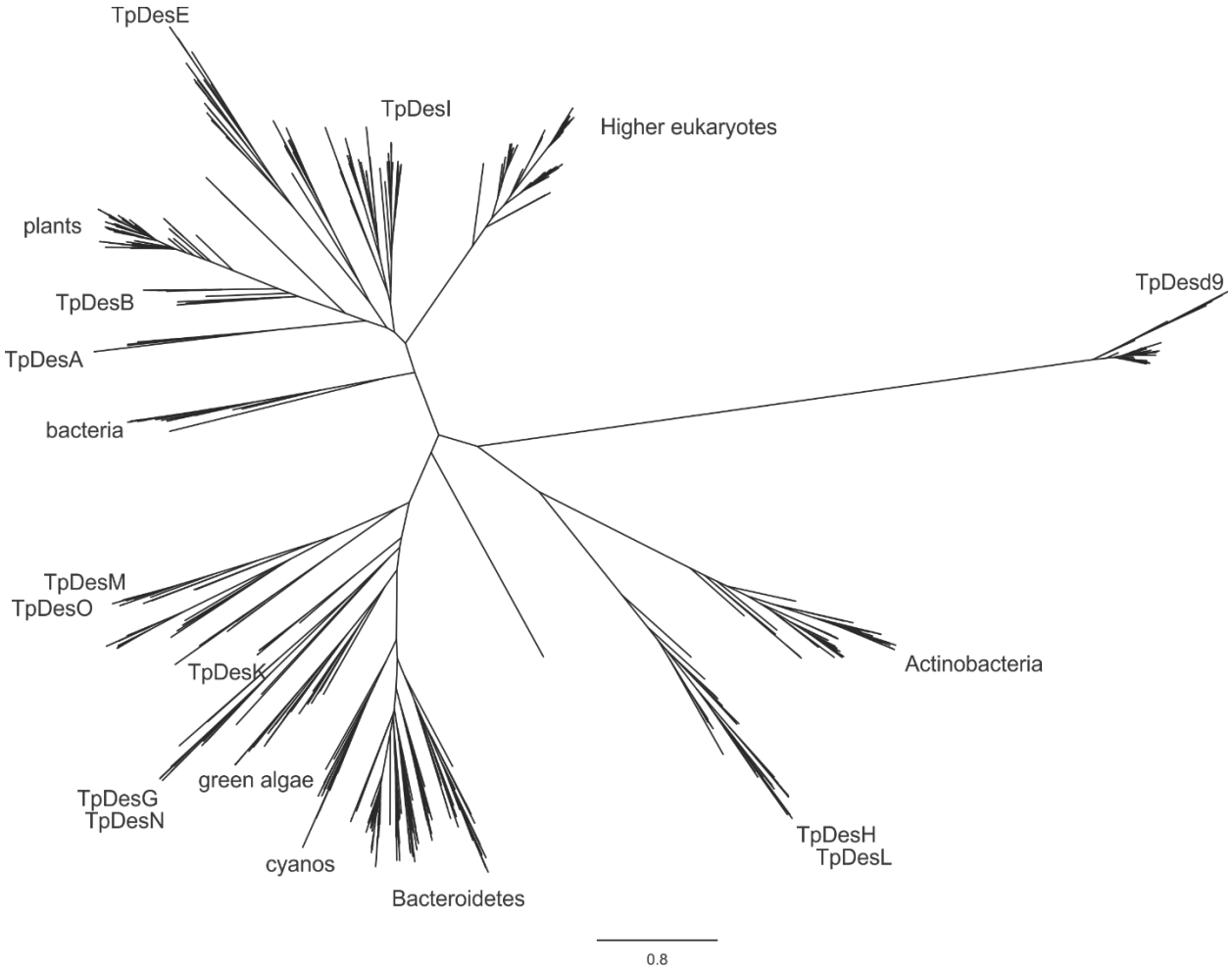


Figure S13 – Phylogeny of *T. pseudonana* desaturases. A phylogenetic tree was constructed from unique sequences of desaturases of *T. pseudonana* and their top blast hits against the whole NCBI database. The ~2000 sequences were aligned using Muscle and the tree constructed using Fasttree.

Table S11 – Growth analysis of feeding acids to *E. coli*.

FA	Name	[FA]	In M9	In LB	Ref
C1	Formic acid	100 mM	Dead	Dead	
		10 mM	+/-	+/-	
C2	Acetic acid	100 mM	Dead	Dead	
		10 mM	+/-	+/-	
C3	Propionic acid	100 mM	Dead	Dead	[51]
		10 mM	+/-	+/-	
C4	Butyric acid	100 mM	Dead	Dead	
		10 mM	+/-	+/-	
C5	Valeric acid	100 mM	Dead	Dead	
		10 mM	++/-	+/-	
C6	Hexanoic acid	100 mM	-/-	-/-	
		10 mM	++/-	+/-	
C7	Heptanoic acid	100 mM	+/-	-/-	
		10 mM	++/-	-/-	
C8	Octanoic acid	100 mM	+/-	+/-	
		10 mM	++/-	-/-	
C9	Nonanoic acid	100 mM	+/-	+	
		10 mM	++/-	+	
C10	Decanoic acid	10 mM	+/--	+	[52]
		1 mM	++/-	+	
C11	Undecanoic acid	10 mM	+/-	+	
		1 mM	++/-	+	
C12	Dodecanoic acid	10 mM	+/-	+	
		1 mM	++/-	+	
C13	Tridecanoic acid	10 mM	+/-, i	+	
		1 mM	++/-, i	+	
C14	Myristic acid	10 mM	+/-, i	+	
		1 mM	++/-, i	+	
C15	Pentadecanoic acid	n/a			
C16	Palmitic acid	10 mM	+/-, i	+	
		1 mM	++/-, i	+	
C17	Heptadecanoic acid	n/a			
C18	Stearic acid	10 mM	+/-, i	+	
		1 mM	++/-, i	+	
control			+	+	

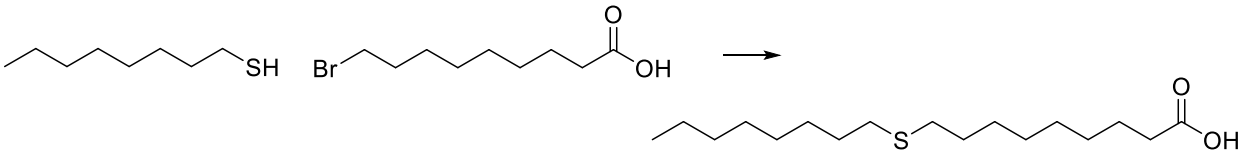
Table S11 - Growth analysis of feeding acids to *E. coli*. Different concentrations of acids were fed to *E. coli* in M9 and LB media. The pH of the solution was checked by pH paper and remained around seven. The data was qualitatively analyzed. Dead = culture cleared completely; -/- severe retardation in growth rate and maximum OD; +/- retardation in growth rate and maximum OD; ++/- grows faster than wildtype but to lesser OD; i = insoluble material in culture

Synthesis

General. Chemicals were obtained from various sources (Fluka, Sigma-Aldrich, Fisher, TCI and Acros). All reactions were carried out under an argon atmosphere in dry solvents and constant magnetic stirring. TLC analysis was performed using silica gel 60 F254 plates (EM Scientific) and visualized using an appropriate stain. Flash chromatography was carried out with Silicycle 60 230-400 mesh. High-res ESI mass spectra were obtained at the UCSD mass spectrometry facility using a Micromass Quattro Ultima Triple Quadrupole MS. Small molecue NMR spectra were obtained on a 400 Mhz Varian Mercury Plus spectrometer, a 500 Mhz JEOL ECA 500 spectrometer and a 500 Mhz Varian VX500 equipped with a XSens 2-channel cold probe. LCMS was run on a Thermo LCQ Deca system at the UCSD mass spectrometry facility using @.

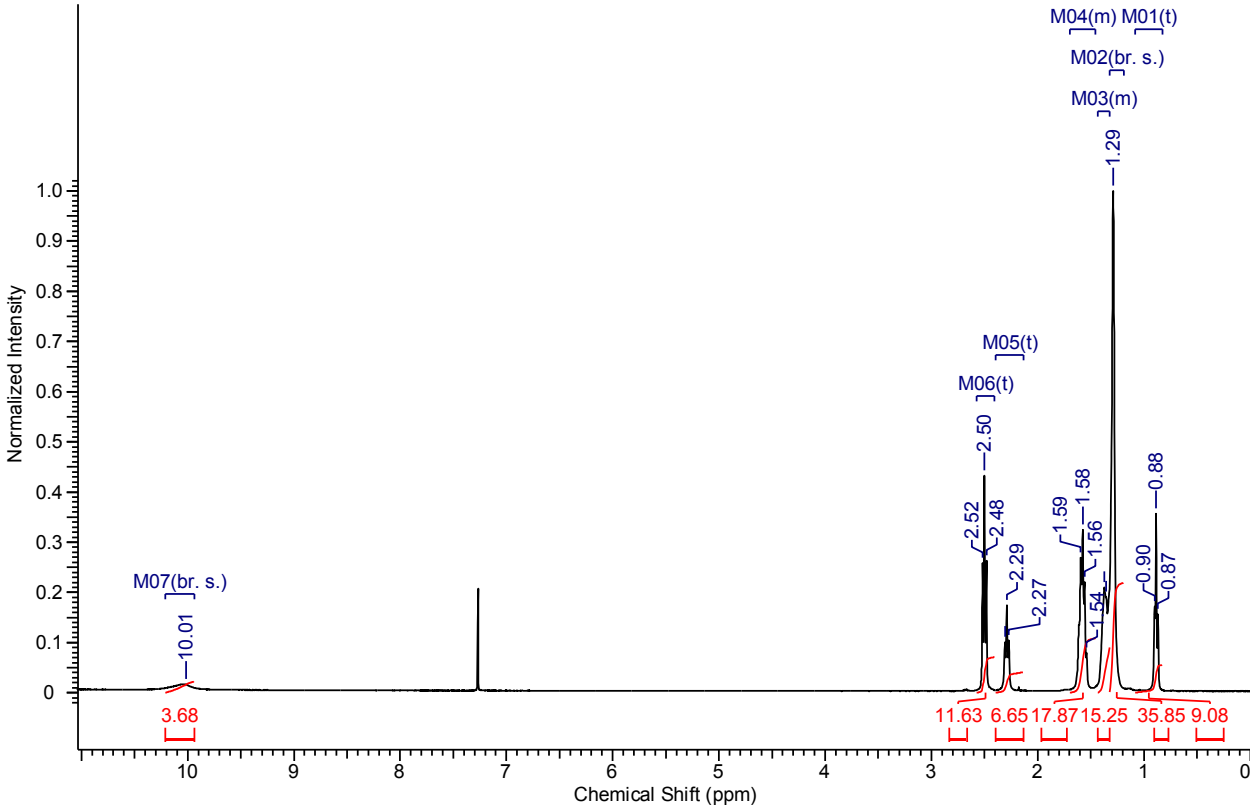
9-thiastearic acid (S9) was obtained from commercial sources.

10-thiastearic acid (S10)[54]

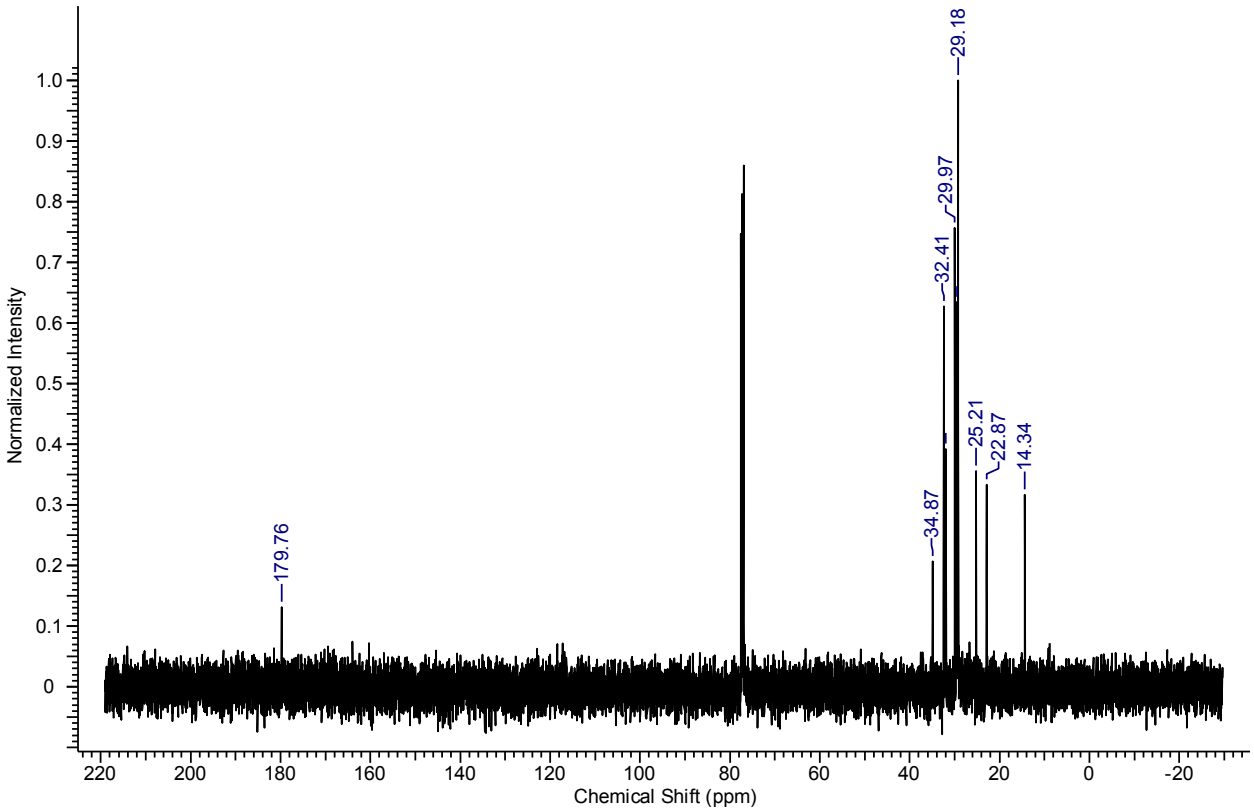


For the synthesis of 10-thiastearic acid we followed the procedure described by Jie et al.[54] Briefly, 9-bromononanoic acid (1 mmol, 237 mg) and octanethiol (1 mmol, 173 µl) were stirred at room temperature under argon. Potassium hydroxide (2 eq.) dissolved in 5 ml ethanol was added slowly. The reaction mixture was heated under reflux for 3 hours. Ethanol was evaporated under vacuum. The residue was taken up in water and acidified. The precipitate which formed was filtered, washed with ice cold water and dried in a desiccator overnight, giving an off-white powder (95%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, *J*=6.42 Hz, 3 H. -CH_3), 1.29 (br. s., 12 H, -CH_2-), 1.32 - 1.43 (m, 6 H, -CH_2-), 1.46 - 1.70 (m, 6 H, -CH_2-) 2.29 (t, *J*=7.33 Hz, 2 H, CH_2-COOH) 2.50 (t, *J*=7.33 Hz, 4 H, -CH_2-S-CH_2-) 10.04 (br. s., 1 H, -COOH). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 179.76, 34.87, 32.42, 32.41, 31.99, 29.97 (2x), 29.62, 29.48 (2x), 29.41, 29.18 (3x), 25.21, 22.87, 14.34. NMR spectra, LCMS chromatograms and mass spectra given below.

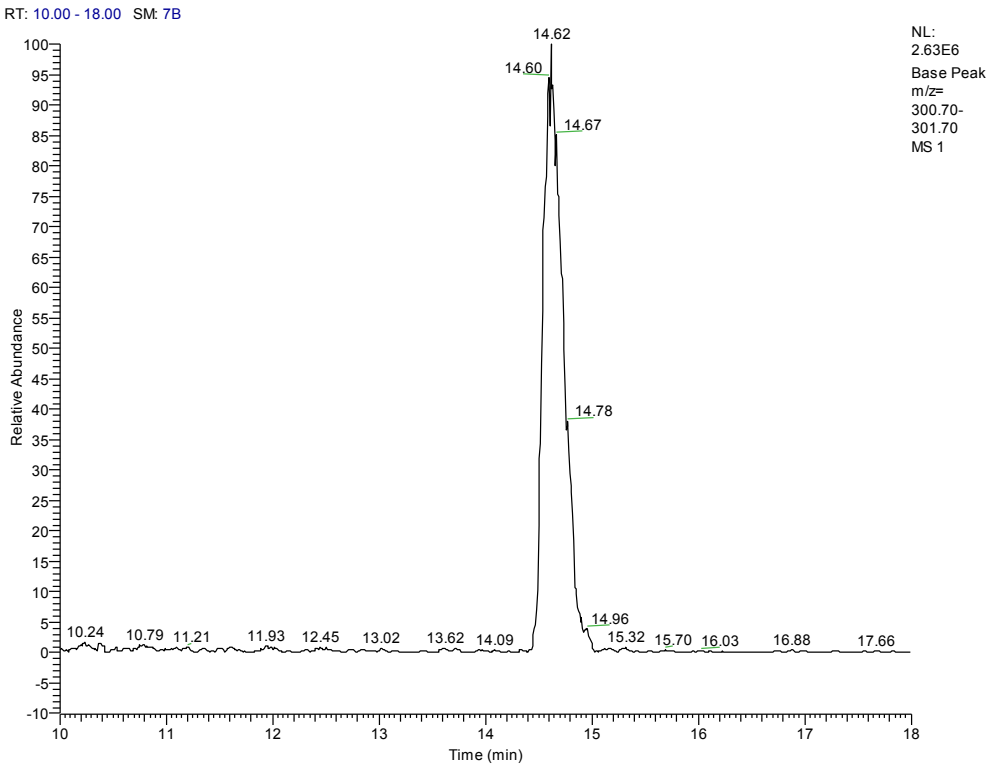
^{1}H-NMR ($CDCl_3$, 400 MHz):



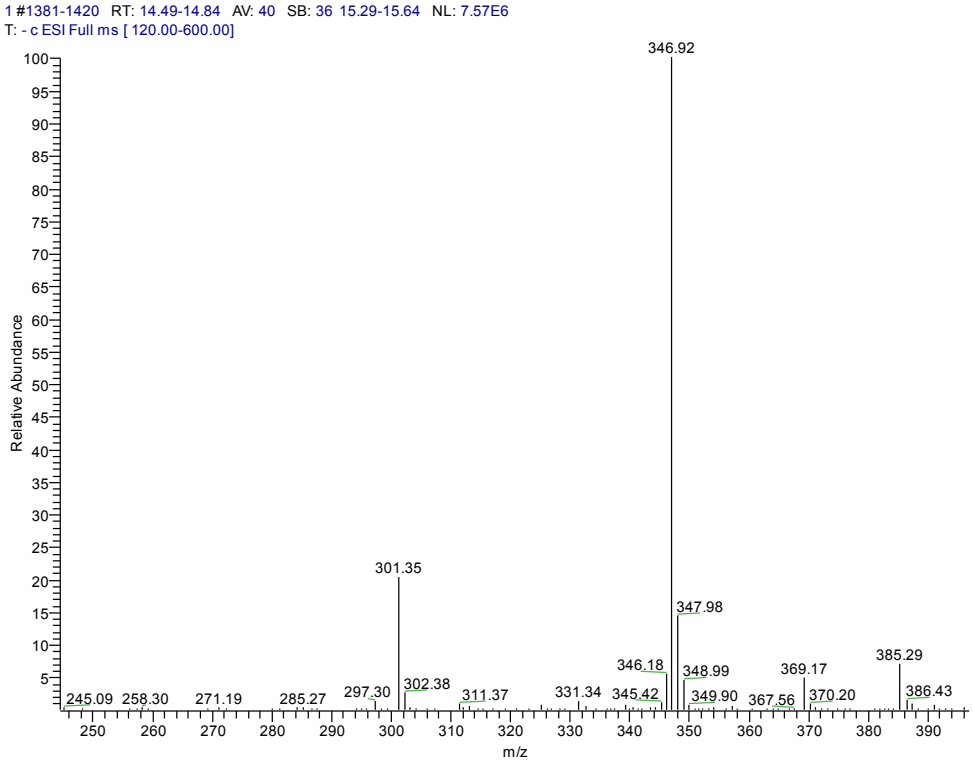
^{13}C-NMR ($CDCl_3$, 100 MHz):



LCMS ESI negative ion mode SIR (expected mass 302.23-1):

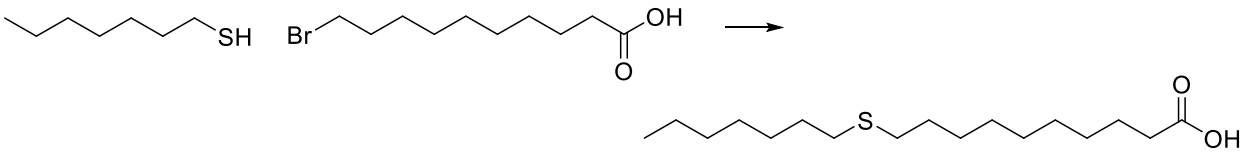


LCMS negative ion mode mass spectrum retention time 14.49-14.84:



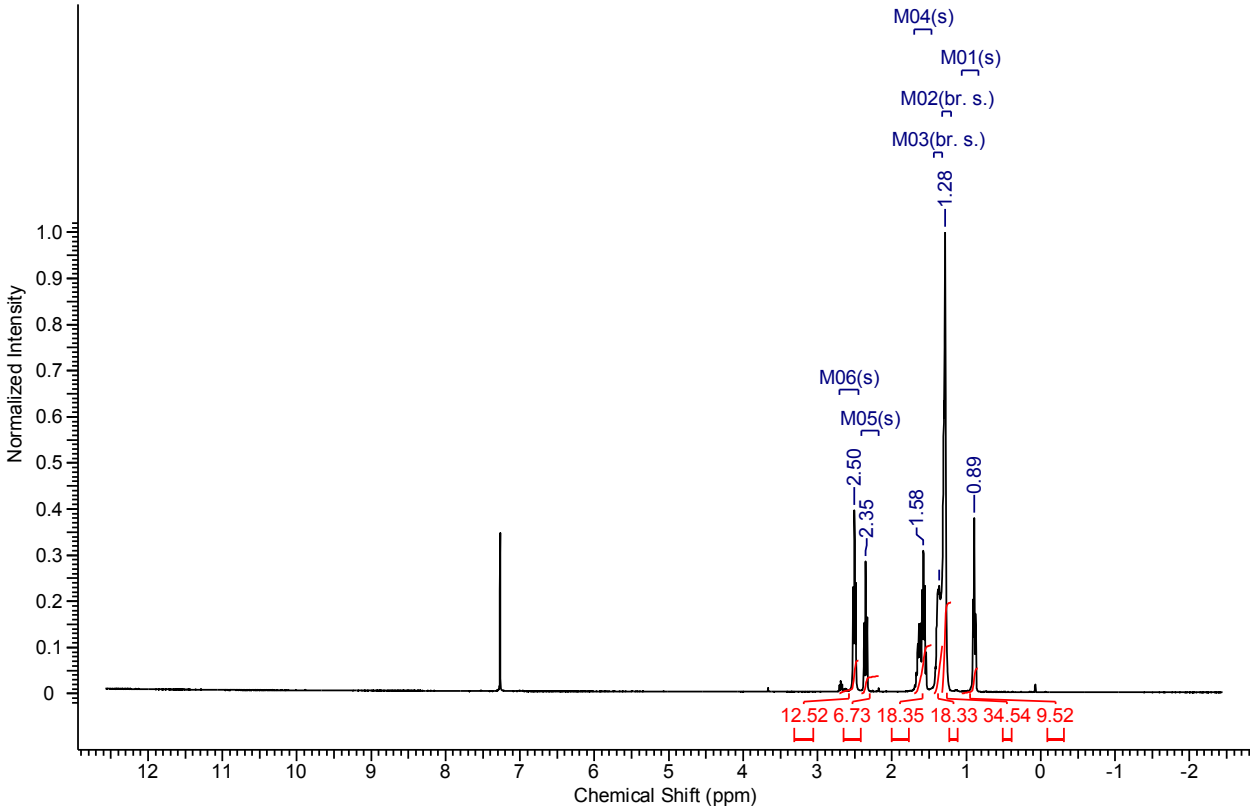
Note that 346.92 corresponds to the expected mass plus formate.

11-thiastearic acid (S11)[54]

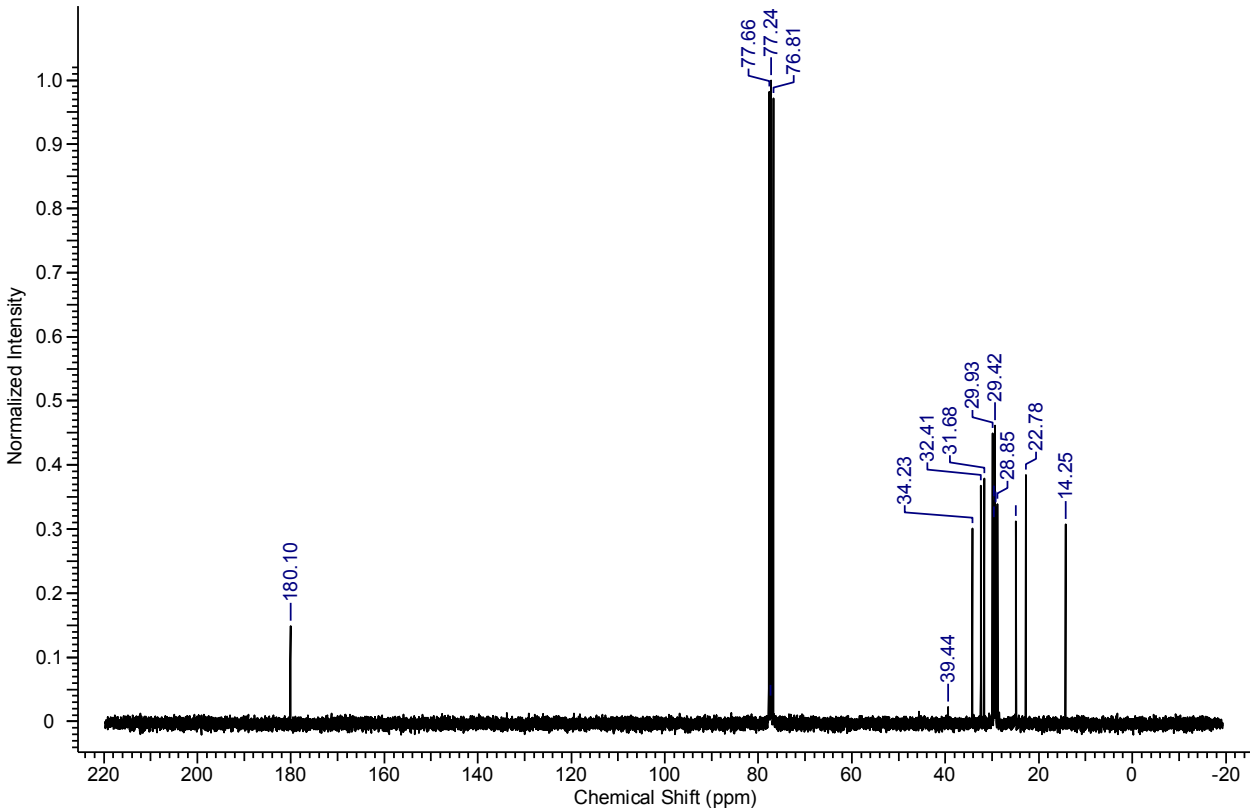


For the synthesis of 11-thiastearic acid we followed the procedure described by Jie et al.[54] Briefly, 10-bromodecanoic acid (1 mmol, 251 mg) and heptanethiol (1 mmol, 155 µl) were stirred at room temperature under argon. Potassium hydroxide (3 eq.) dissolved in 5 ml ethanol was added slowly. The reaction mixture was heated under reflux for 3 hours. Ethanol was evaporated under vacuum. The residue was taken up in water and acidified. The precipitate which formed was filtered, washed with water and dried in a desiccator overnight, giving an off-white solid (95%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.89 (s, 3 H, $-CH_3$) 1.28 (br. s., 12 H, $-CH_2-$) 1.37 (br. s., 6 H, $-CH_2-$) 1.58 (s, 6 H, $-CH_2-$) 2.35 (s, 2 H, $-CH_2-COOH$) 2.50 (s, 4 H, $-CH_2-S-CH_2-$). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 180.10, 34.23, 32.41, 32.39, 31.68, 29.93 (2x), 29.65, 29.56, 29.42 (2x), 29.25, 29.15, 28.85, 24.87, 22.78, 14.25. NMR spectra, LCMS chromatograms and mass spectra given below.

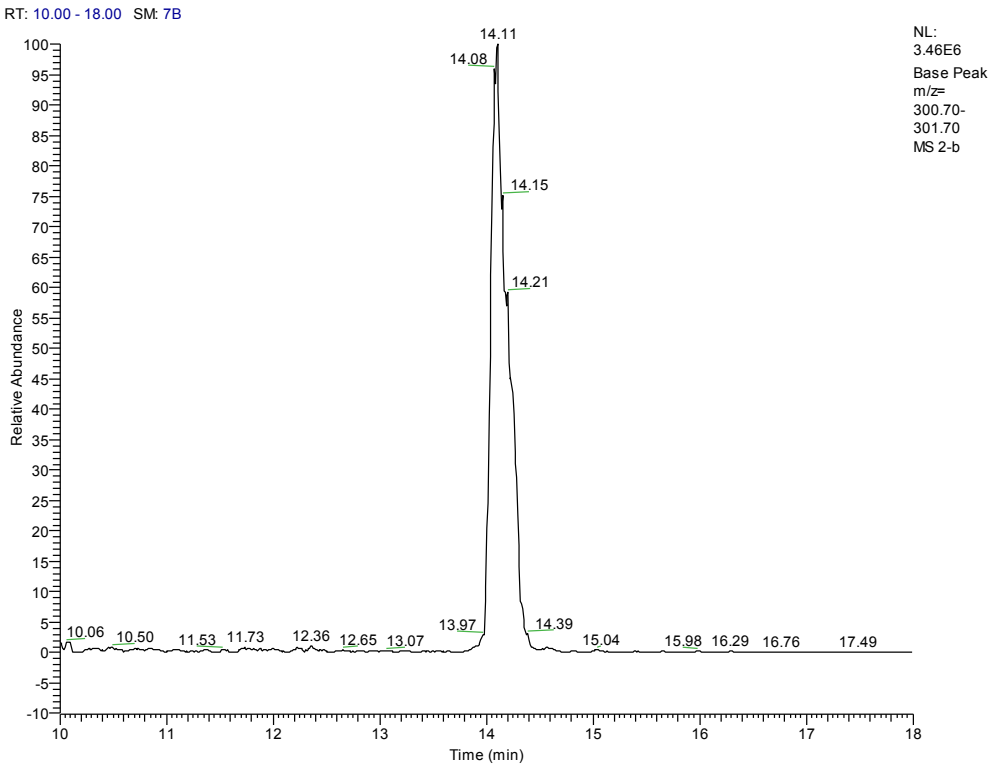
^{1}H-NMR ($CDCl_3$, 400 MHz):



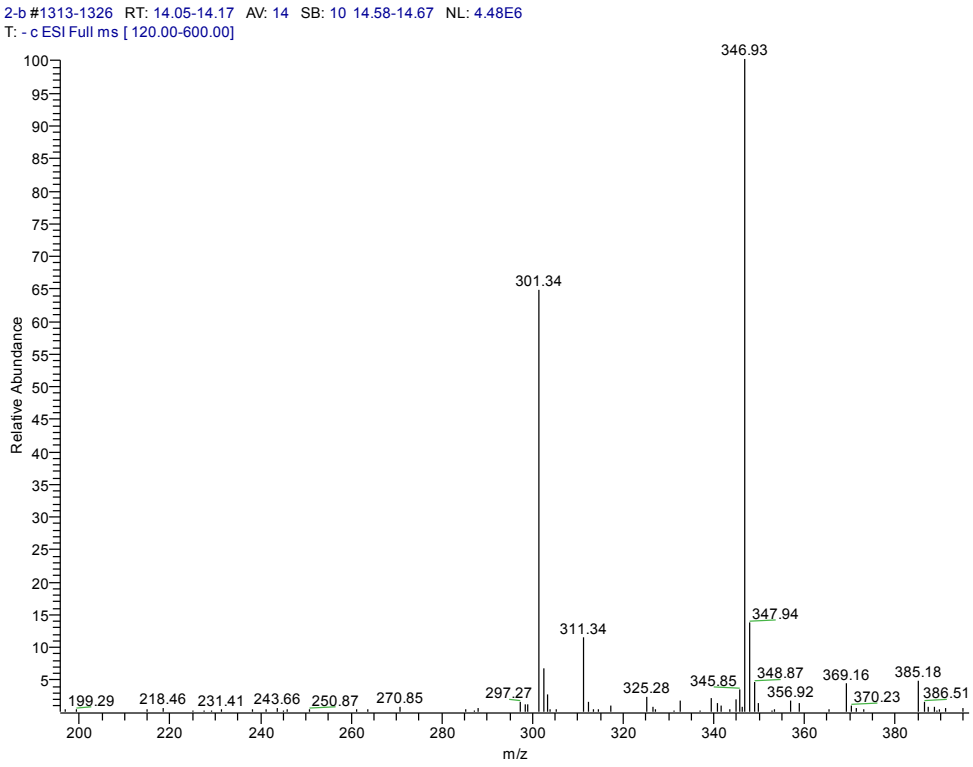
^{13}C-NMR ($CDCl_3$, 100 MHz):



LCMS ESI negative ion mode SIR (expected mass 302.23-1):



LCMS negative ion mode mass spectrum retention time 14.05-14.17:



Note that 346.92 corresponds to the expected mass plus formate.

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