Electronic Supplementary Material (ESI) for Molecular BioSystems.

Electronic Supplementary Information

Production of Long Chain Alcohols and Alkanes Upon Coexpression of an Acyl-ACP Reductase and Aldehyde-Deformylating Oxygenase with a Bacterial Type-I Fatty Acid Synthase in *E. coli*

Dan Coursolle,[a] Jiazhang Lian,[a] John Shanklin,[b] and Huimin Zhao*[ac]

[a] Department of Chemical and Biomolecular Engineering, Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

[b] Brookhaven National Laboratory, P.O. Box 5000, 50 Bell Avenue, Building 463, Upton, NY 11973, USA.

[c] Departments of Chemistry, Biochemistry and Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

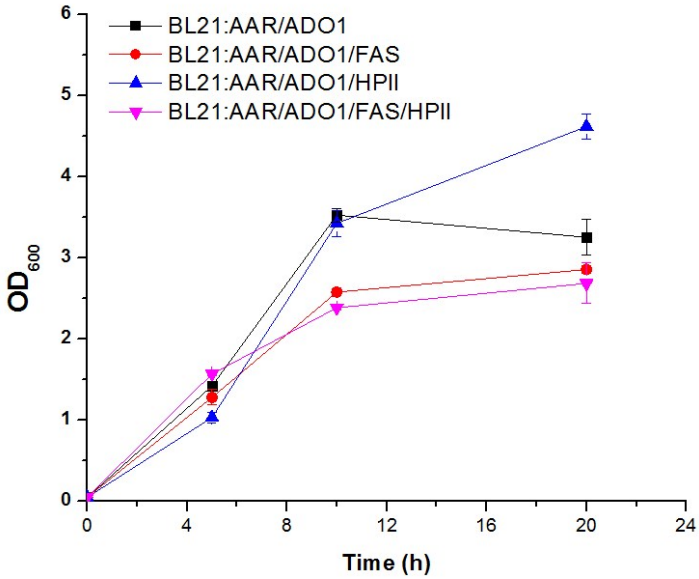
*To whom correspondence should be addressed. Phone: (217) 333-2631. Fax: (217) 333-5052. E-mail: zhao5@illinois.edu.

Table S1. Primers used in this study

Origin	Locus tag	Primer pair sequences
Synechococcus elongatus PCC 7942	Synpcc7942_1594	NNNccatggATGTTCGGTCTTATCGGTCATC
		NNNNNNNNNgcggccgcTCAAATTGCCAATGCCAAGG
Cyanothece sp. Strain ATCC 51142	cce_1430	NNNccatggATGTTTGGTTTAATTGGTCATC
		NNNNNNNNNgcggccgcTTACCAACTCAACAACGGTTG
Synechocystis sp. PCC 6803	SYNPCCP_2249	NNNccatggATGTTTGGTCTTATTGGTCATC
		NNNNNNNNNgcggccgcCTAAAGAGCTACTAAAGGGC
Cyanothece sp. PCC 8801	PCC8801_0872	NNNggatccATGTTTGGTCTGATCGGTCATC
		NNNNNNNNNgcggccgcTTACCAACTCAATAAAGGTCG
Cyanothece sp. PCC 8802	Cyan8802_0898	NNNggatccATGTTTGGTCTGATCGGTCATC
		NNNNNNNNNgcggccgcTTACCAACTCAATAAAGGTCG
Cyanothece sp. PCC 7425	Cyan7425_0399	NNNggatccATGTTCGGATTAATTGGCCATC
		NNNNNNNNNgcggccgcCTACTGAAATAACAGGGGTTG
Synechococcus elongatus PCC 7942	Synpcc7942_1593	NNNagatctATGCCGCAGCTTGAAGCCAGC
		NNNcctaggTCAAACGGCCGCAAGGCCATAG
Cyanothece sp. Strain ATCC 51142	cce_0778	NNNagatctATGCAAGAGCTTGCTTTACGC
		NNNcctaggTTAAGCAGCCCGTAGCCCGT
Synechocystis sp. PCC 6803	SYNPCCP_2250	NNNagatctATGCCCGAGCTTGCTGTCCG
		NNNcctaggCTAGACTCCGGCCAAACCGT
Cyanothece sp. PCC 8801	PCC8801_0455	NNNagatctATGCAAGAGCTTGTCCAACGC
		NNNcctaggTTAAGCTGCCACTAAACCATG
Cyanothece sp. PCC 8802	Cyan8802_0468	NNNagatctATGCAAGAGCTTGTCCAACG
		NNNcctaggTTAAGCTGCCACTAAGCCATG
Cyanothece sp. PCC 7425	Cyan7425_2986	NNNagatctATGTCTGATTGCGCCACGAA
		NNNcctaggCTAAGGCGTAACCGCCTGTA
	Cyan7425_0398	NNNcctaggTTATGCCGCAGAGAGGCCGTA
		NNNNNNNNNcatatgATGCCTCAAGTGCAGTCCCCA

Figure S1

A



B

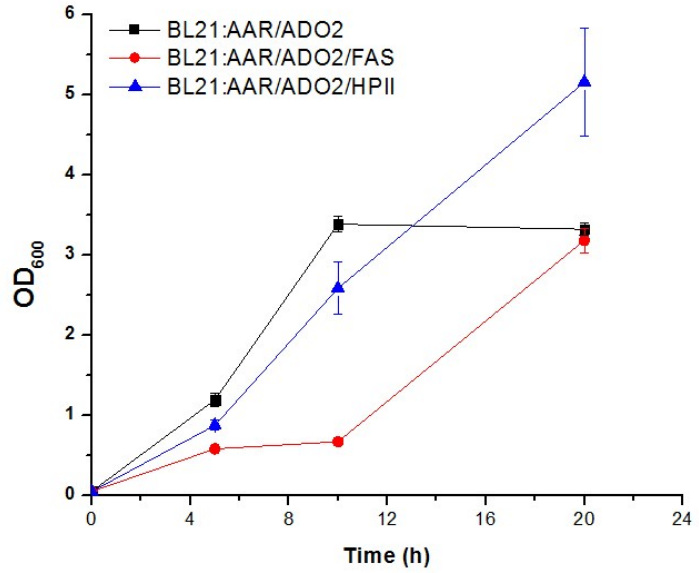


Figure S1. Growth curve of recombinant alkane-producing *E. coli* strains harboring ADO1 (A) or ADO2 (B). Cells were pre-cultured in LB medium overnight and inoculated into fresh modified M9 medium containing 3% glucose. After culture for 6 h, 0.5 mM IPTG was added to induce the expression of heterologous genes. Samples were taken at the mentioned time points and diluted 5-10 folds to measure the cell densities (absorbance at 600 nm) using a Biotek Synergy 2 Multi-Mode Microplate Reader (Winooski, VT).

Figure S2

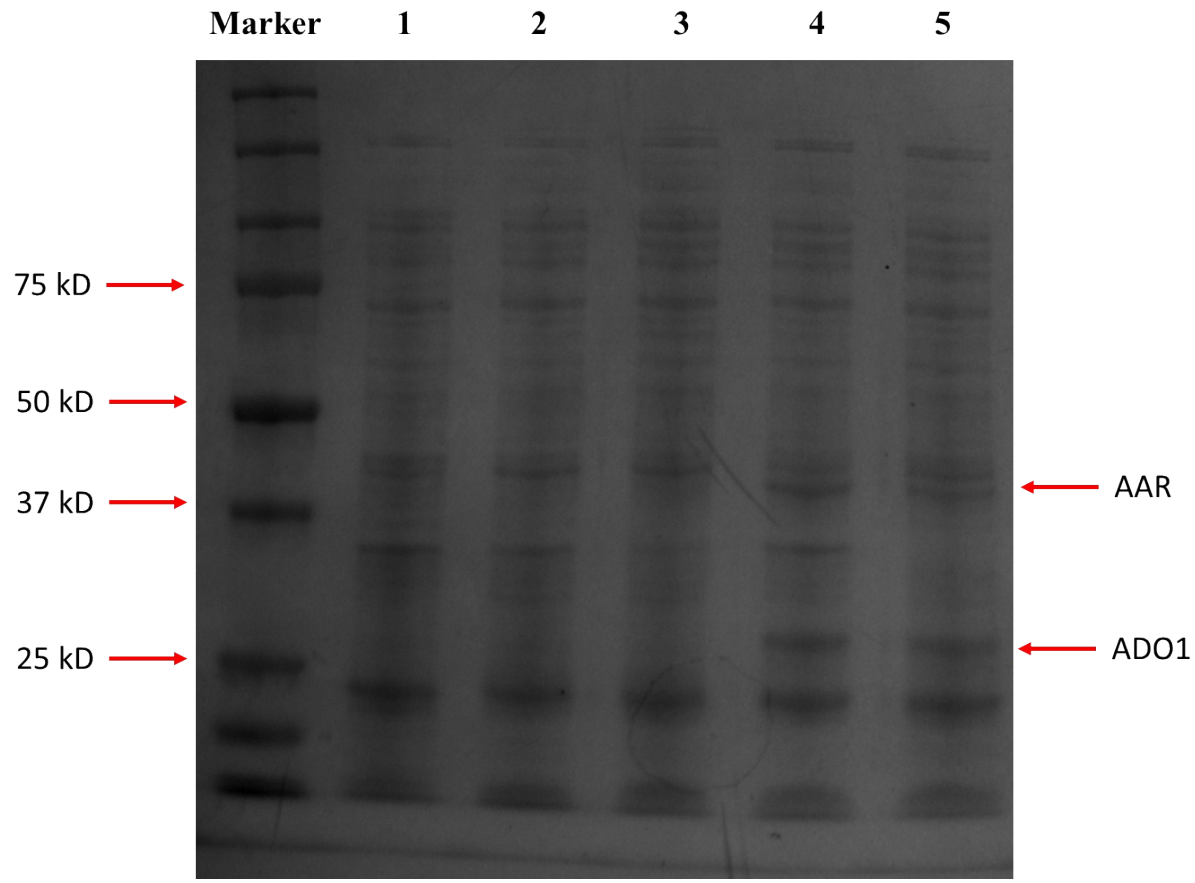
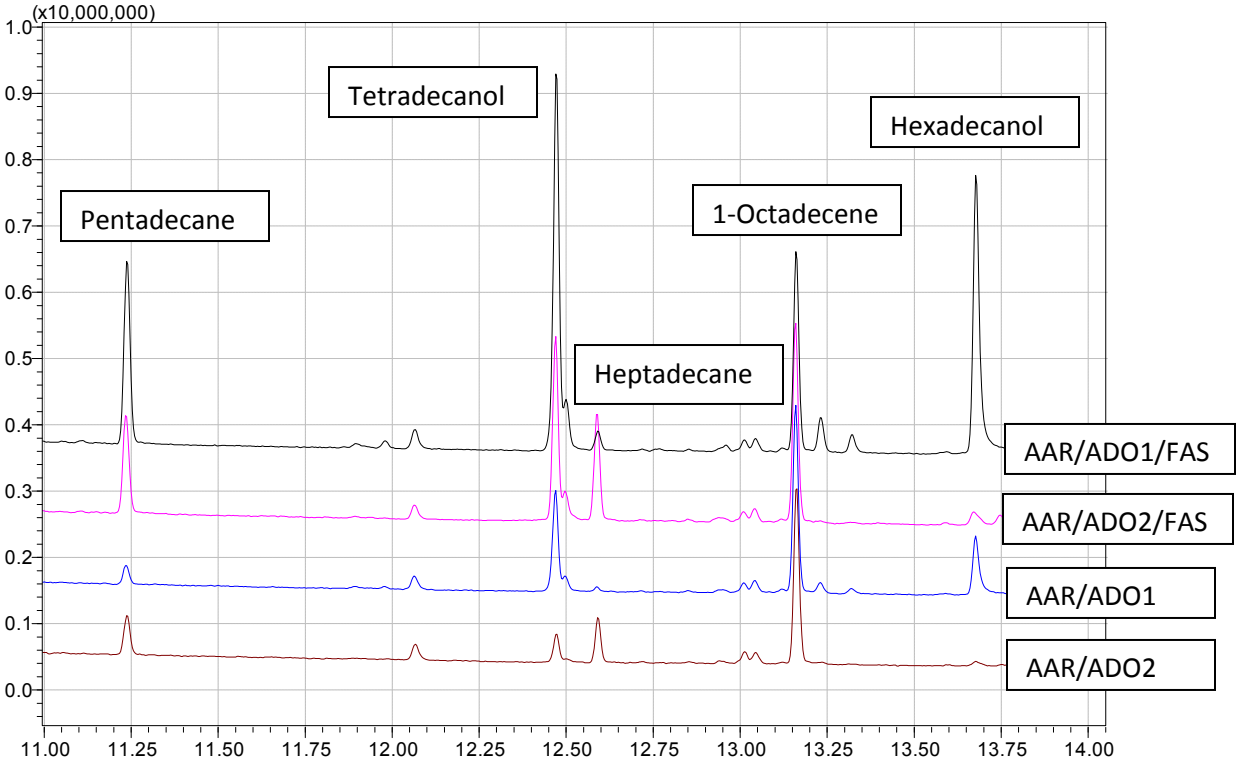


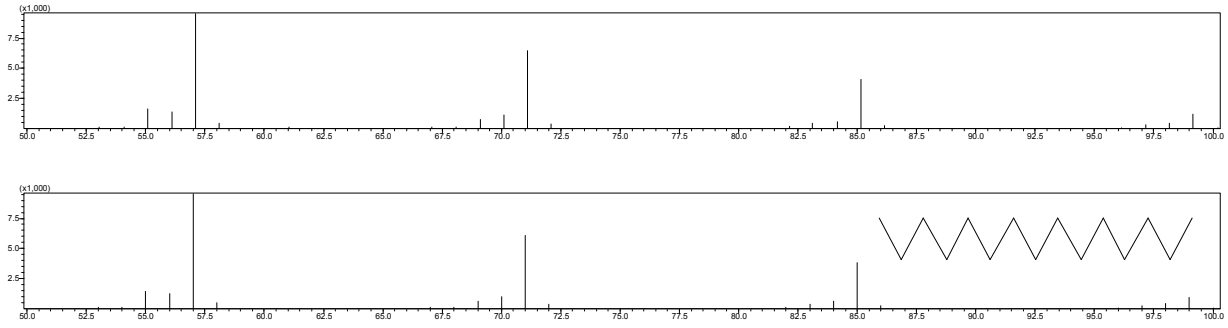
Figure S2. SDS-PAGE analysis of the expression of heterlogous proteins. Cells were pre-cultured in LB medium overnight and inoculated into 10 mL fresh modified M9 medium containing 3% glucose. After additional culture for 6 h, 0.5 mM IPTG was added to induce the expression of heterologous genes. After induction for16h, cells were harvested by centrifugation, washed twice in ddH_2O, and resuspended in 1 mL 10 mM Tris-HCl pH8.0 buffer. Then the cells were disrupted by sonication using a Sonic Dismembrator Model 500 from Fisher Scientific until the solution became clear. Cell debris were removed by centrifugation at 15,000 rpm for 10 min at 4^{o}C, and the resulting supernatants containing all the soluble proteins were analyzed by SDS-PAGE (12% Gel). Lane 1, BL21(DE3); Lane 2, BL21:AAR/ADO/FAS withour IPTG induction; Lane 3: BL21:AAR/ADO/FAS/HPII; Lane 4: BL21:AAR/ADO/FAS; Lane 5: BL21:AAR/ADO.

Figure S3

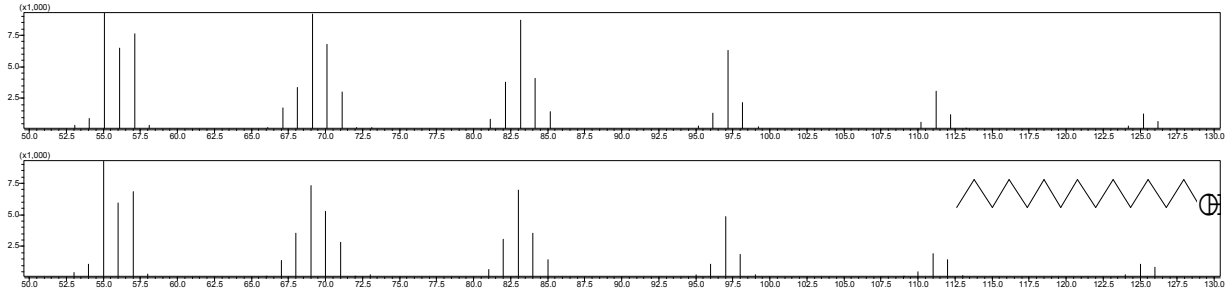
A



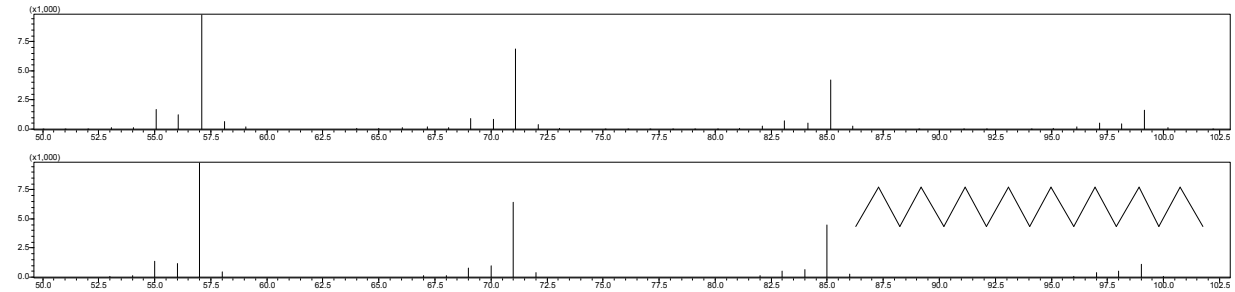
B



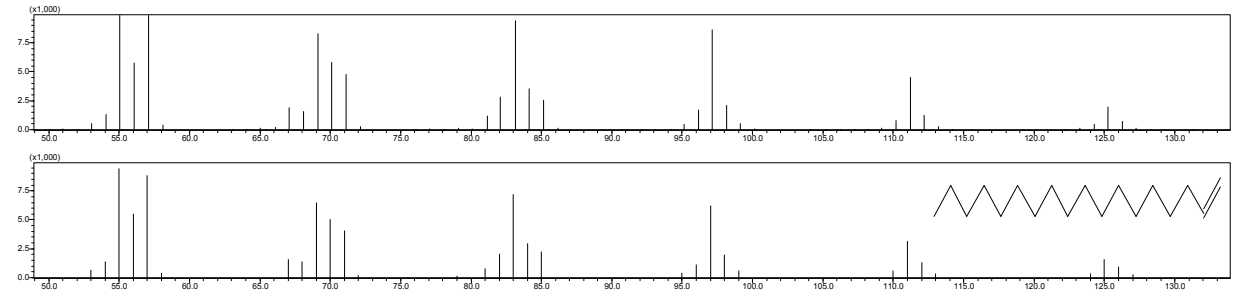
C



D



E



F

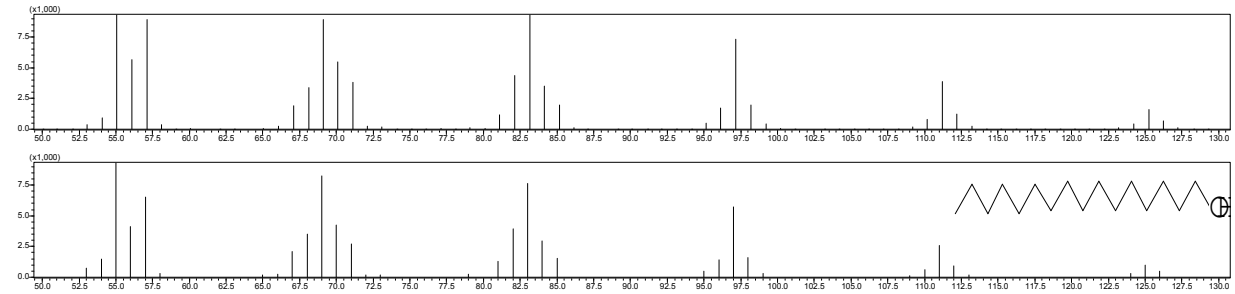
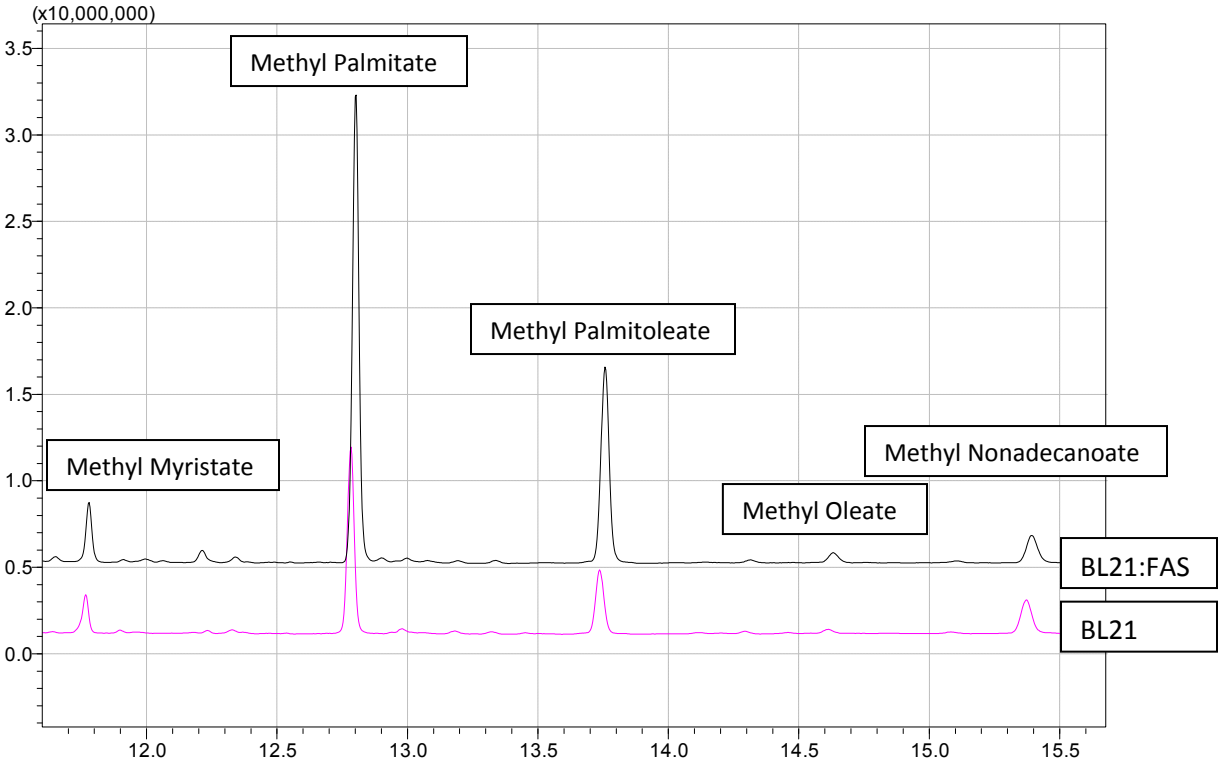


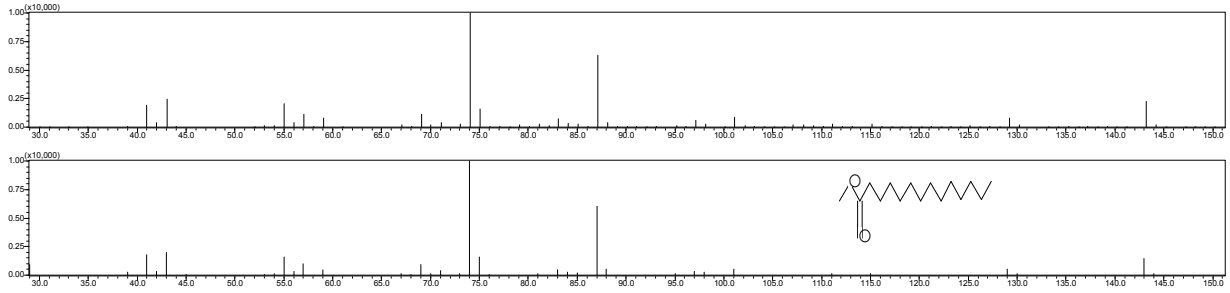
Figure S3. GC/MS traces of the product profiles of BL21:AAR/ADO1, BL21:AAR/ADO2, BL21:AAR/ADO1/FAS, and BL21:AAR/ADO2/FAS. The GC profiles (A) highlighted the major products (pentadecane, tetradecanol, heptadecane, and hexadecanol) and the internal standard (1-octadecene). B, C, D, E, and F showed the MS fragmentation pattern of pentadecane, tetradecanol, heptadecane, 1-octadecene, and hexadecanol, respectively, with the top panel showing the target and the bottom showing the corresponding standard.

Figure S4

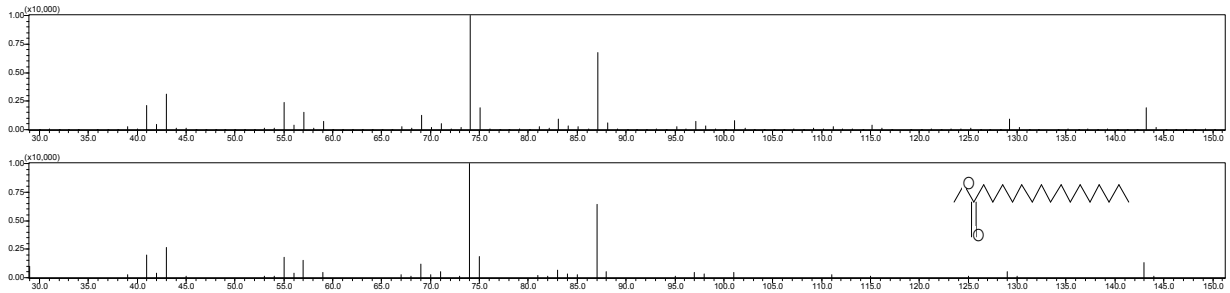
A



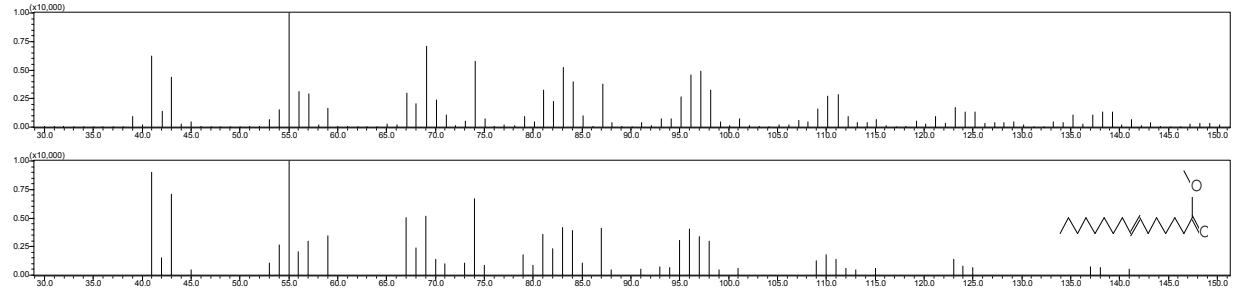
B



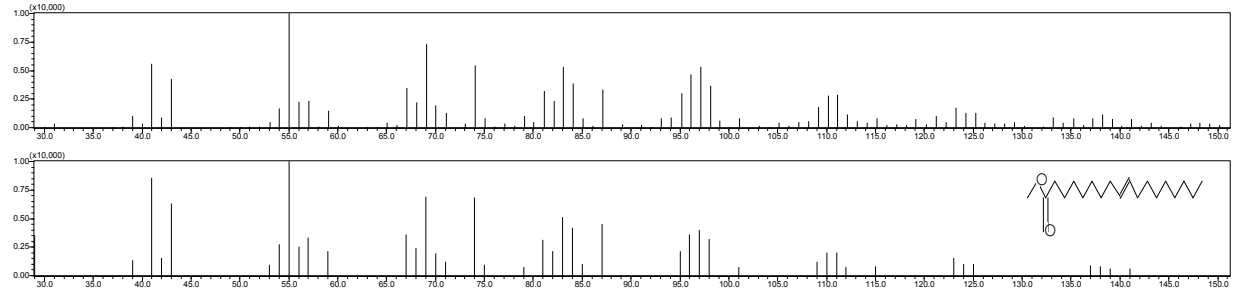
C



D



E



F

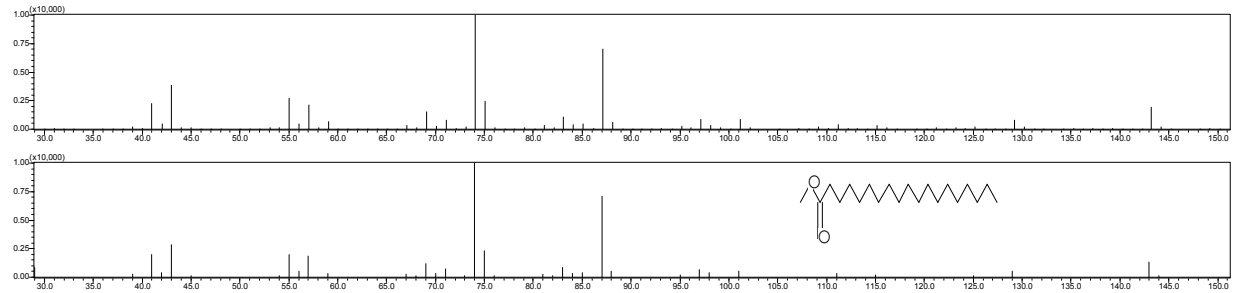


Figure S4. GC/MS traces of the product profiles of BL21: FAS. The GC profiles (A) highlighted the major fatty acids (myristic acid, palmitic acid, palmitoleic acid, and oleic acid) and the internal standard (nonadecanoic acid) in the methyl ester form. B, C, D, E, and F showed the MS fragmentation pattern of the methyl ester of myristic acid, palmitic acid, palmitoleic acid, oleic acid, and nonadecanoic acid, respectively, with the top panel showing the target and the bottom showing the corresponding standard.