Materials and Methods:

ADC and AAR cloning

The *aar* and *adc* genes from *Synechococcus elongates* PCC7942 (accession numbers YP_400611 and YP_400610, respectively) were separately constructed from synthesized oligos that were designed using Helix Systems DNAWorks (http://helixweb.nih.gov/dnaworks). Both protein coding sequences were flanked by the BioBricks Prefix (5'-GAATTCGCGGCCGCTTCTAG-3') at the 5' end, and the BioBricks Suffix (5'-TAATGATACTAGTAGCGGCCGCTGCAGGAAGAAAC –3') at the 3' end. Oligo design was performed using the following DNAWorks parameters: codon optimization for *E. coli* standard, oligo length 60 nt, annealing temperature 58 ± 1°C, oligo concentration $1.00*10^{-7}$ M, sodium concentration $5.00*10^{-2}$ M, Mg^{2+} concentration $2.00*10^{-3}$ M, codon frequency threshold 10%, repeat threshold 8 nt, mispriming threshold 8/18 (6 exact) nt. Both gene sequences were designed to avoid the recognition sites of the restriction enzymes BamH1, BglII, EcoRI, KpnI, NcoI, NdeI, NheI, NotI, PatI, SacI, SalI, SpeI, XbaI, XhoI, and XmaI.

The oligos were diluted to 100 μM and 5 μl of each were combined into an Oligo Mastermix. Oligos were assembled by overlap extension (OE) PCR using the following reaction mixture: 1 μL of Oligo Mastermix, 1 μL 25 mM dNTPs, 10 μL Phusion HF buffer, 0.5 μL of 100 μM 5' oligo, 0.5 μL of 100 μM 3' oligo, 0.5 μL Phusion polymerase, and 36.5 μL diH_2O. This amplification was performed using the following PCR program: 98°C for 30 sec; and then 29 cycles of: 98°C for 10 sec, 63°C for 10 sec, and 72°C for 30 sec per kb gene length; followed by a final extension at 72°C for 5 min. The product from the overlap extension PCR reaction was directly used in a second round of PCR using the 5' and 3' oligos as sense and antisense primers. The reaction mixture for this amplification was composed of: 1 μL OE PCR reaction product, 1 μL of 25 mM dNTPs, 10 μL Phusion HF buffer, 0.5 μL of 100 μM 5' oligo, 0.5 μL of 100 μM 3' oligo, 0.5 μL Phusion polymerase, and 36.5 μL diH_2O. The PCR program used was the same as for the OE PCR.

The *aar* and *adc* genes were serially cloned into BBa_K314100, a high copy number expression vector containing a constitutive promoter, part BBa_J23100, and an RBS, part BBa_B0034. This was accomplished by digestion of both the vector and constructed gene with the restriction enzymes EcoR1 and PstI, and subsequent ligation of these fragments using T7 DNA Ligase (6). *adc* was cloned 5' of *aar.* This AAR and ADC expression vector is part BBa_K590025.

FabH2 cloning

The *fabH2* gene from *Bacillus subtilis* (accession number O07600) was constructed from oligos that were designed using Helix Systems DNAWorks (http://helixweb.nih.gov/dnaworks). The FabH2 protein coding sequence was flanked by the BioBricks Prefix (5'- GAATTCGCGG CCGCTTCTAG- 3') at the 5' end, and the BioBricks suffix (5'-TAATGATACT AGTAGCGGCC GCTGCAGGAA GAAAC – 3') at the 3' end. In addition, a glycine-serine linker followed by a FLAG tag was added to the C terminus of the protein (GGSGGS DYKDDDDK). Oligo design was conducted using the same DNAWorks parameters as for *aar* and *adc* (described above), except oligos were designed to have a length of 50 bp. Oligo assembly was performed using the same protocol as used for *aar* and *adc*.

fabH2 was cloned into BBa_K314103, a low copy number expression vector that contains an IPTG inducible promoter, part BBa_R0011, and an RBS, part BBa_B0034. This was accomplished by digestion of both the vector and constructed *fabH2* gene with the restriction enzymes EcoR1 and PstI, and subsequent ligation of these fragments using T7 DNA Ligase (6). This FabH2 expression vector is part BBa_K590064.

Constructs and strains

All constructs were tested in *E. coli* BL21(DE3). In order to control for antibiotic resistance in experiments conducted with AAR and ADC expression, but without FabH2 expression, the AAR and ADC expression vector was co-transformed with a pSB3K3 low copy number plasmid expressing GFP from an IPTG-inducible promoter. This GFP expression part was constructed by cloning GFP (part BBa_E0040) into an IPTG-inducible expression plasmid (part BBa_K314103).

Growth conditions

All strains were growth in 50 mL of Terrific Broth media with 50 mg/L kanamycin sulfate and 34 mg/L chloramphenicol at 37°C for 24 hours with shaking in 250 mL baffled flasks. Cells were then pelleted and resuspended in 750 µL of modified M9 glucose media, resulting in a culture with an OD_{600} of approximately 10. The modified M9 glucose media was composed of: 3% glucose, 42 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.5 mM NaCl, 37 mM NH_4Cl, 200 mM Bis-Tris (pH 7.25), 0.1% TritonX-100, 0.3 mM thiamine, 0.1 mM $FeCl_3$, 1 mM $MgSO_4$, 50 mg/L kanamycin sulfate, 34 mg/L chloramphenicol, 10 µM IPTG, and 10 mg/L undecane as an internal standard. For media supplemented with propanoate, 6.5 mM propanoate was added to the production media. The resuspended culture was transferred to a 15x150 mm glass culture tube, which was then sealed. This culture was incubated at 37°C with shaking for 24 hours, after which

hydrocarbons were extracted from the media by the addition of 750 µL ethyl acetate. This mixture of culture and ethyl acetate was vortexed, transferred into an Eppendorf microcentrifuge tube, and centrifuged at 14,000 rpm for one minute. The resulting organic layer was removed and analyzed using gas-chromatography coupled with mass-spectrometry (GC-MS).

Alkane detection:

The organic layer of the ethyl acetate extraction was directly analyzed using an Agilent 5975 GC-MS. Peak identification was conducted by comparing MS signals to the NIST MS library using NIST MS search 2.0 (Supplemental Figure 3), as well as by comparing elution times to that of product standards. All quantification was performed on the m/z 57 ion extractions, and all raw integration signals were normalized to the internal undecane standard signal before yield calculation. To generate a standard curve, alkane extraction and analysis were performed on a cell culture that was incapable of generating alkanes but had been spiked with tridecane, tetradecane, pentadecane, hexadecane, and heptadecane. To control for antibiotic resistance, this cell culture was co-transformed with a low copy number pSB3k3 plasmid encoding IPTG-inducible GFP and a high copy number pSB1C3 plasmid constitutively expressing GFP. These GFP plasmids were constructed by cloning GFP, part BBa_E0040, into the expression vectors BBa_K314103, and BBa_K314100, respectively. The standard curves (Supplemental Figure 4) exhibited nonlinear response and therefore were fit to a power curve function. All microbially produced alkanes in this report fell within the range of the product concentrations used in the standard curve.

GC Parameters:

Injection volume	1 µL
Inlet heater temperature	250°C
Inlet pressure	7.07 psi
Total inlet flow	0.5 mL/min
Septum purge flow mode	Standard
Inlet Mode	Splitless
Column	Agilent 19091S-433: 325°C: 30 m x 250 µm x 0.25µm
Column flow rate	1 mL/min
Column Pressure	7.07 psi
Average velocity	36.262cm/sec
Holdup Time	1.3789

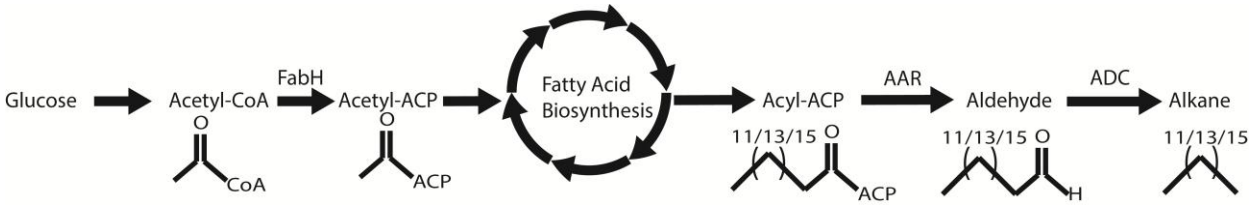
Initial oven temperature	40°C for 1min
Ramp 1	Heat 15°C/min until 100°C
Ramp 2	Heat 25°C/min until 320°C, hold for two min

Supplemental References:

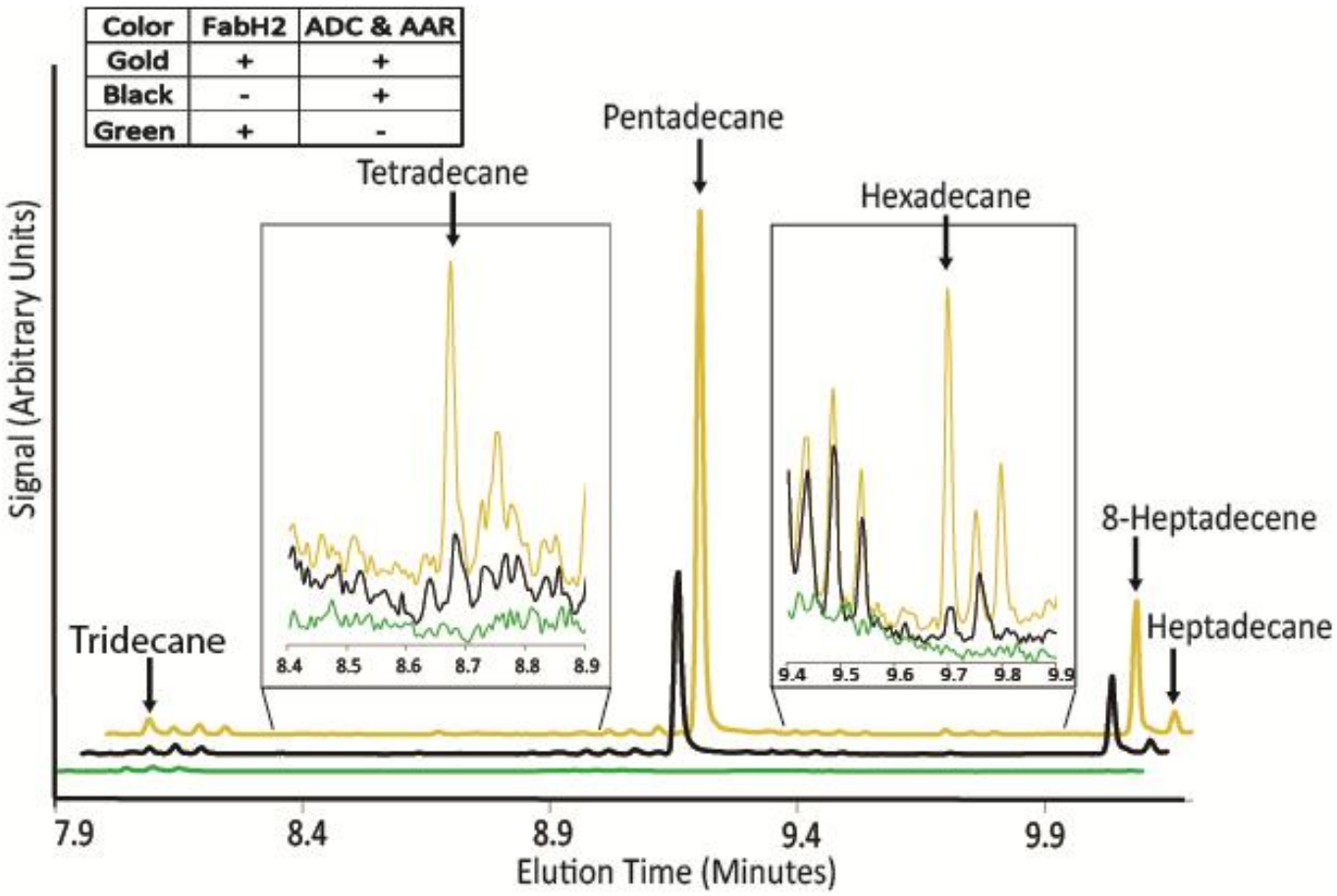
6. Cohen, H. N., Chang, A. C., Boyer, H. W., Helling, R. B., **(1973)** Construction of Biologically Functional Bacterial Plasmids *In Vitro* 70, 3240-3244

	0 mM Propanoate				**6.5 mM Propanoate**			
FabH2 (part BBa_K590064)	-		+		-		+	
IPTG concentration	0 µM	10 µM	0 µM	10 µM	0 µM	10 µM	0 µM	10 µM
Tridecane (C13)	5.9 ± 0.3	6.3 ± 0.2	14.7 ± 1.2	14.9 ± 1.2	6.8 ± 0.3	6.4 ± 0.7	11.0 ± 0.6	13.6 ± 1.4
Tetradeacane (C14)	trace	trace	4.0 ± 0.6	3.7 ± 0.2	3.4 ± 0.2	3.5 ± 0.3	9.8 ± 0.3	14.3 ± 1.9
Pentadecane (C15)	24.8 ± 2.5	23.7 ± 2.1	48.6 ± 5.9	45.2 ± 7.3	23.7 ± 1.3	24.2 ± 1.6	46.1 ± 1.6	41.8 ± 5.8
Hexadecane (C16)	trace	trace	1.2 ± 0.1	1.2 ± 0.3	2.1 ± 0.5	1.8 ± 0.1	8.4 ± 0.2	11.9 ± 2.4
8-Heptadecene (C17)	7.7 ± 1.1	7.4 ± 1.5	16.0 ± 3.5	13.2 ± 3.2	7.1 ± 0.7	8.2 ± 0.4	18.7 ± 0.6	13.5 ± 2.7
Heptadecane (C17)	2.2 ± 0.3	2.1 ± 0.4	3.3 ± 0.7	3.0 ± 0.5	2.1 ± 0.3	2.1 ± 0.1	3.9 ± 0.2	3.2 ± 0.4
Total yield	40.6 ± 3.6	39.4 ± 3.8	87.8 ± 9.2	81.3 ± 12.4	45.1 ± 1.9	46.3 ± 2.1	98.0 ± 3.3	98.3 ± 13.7

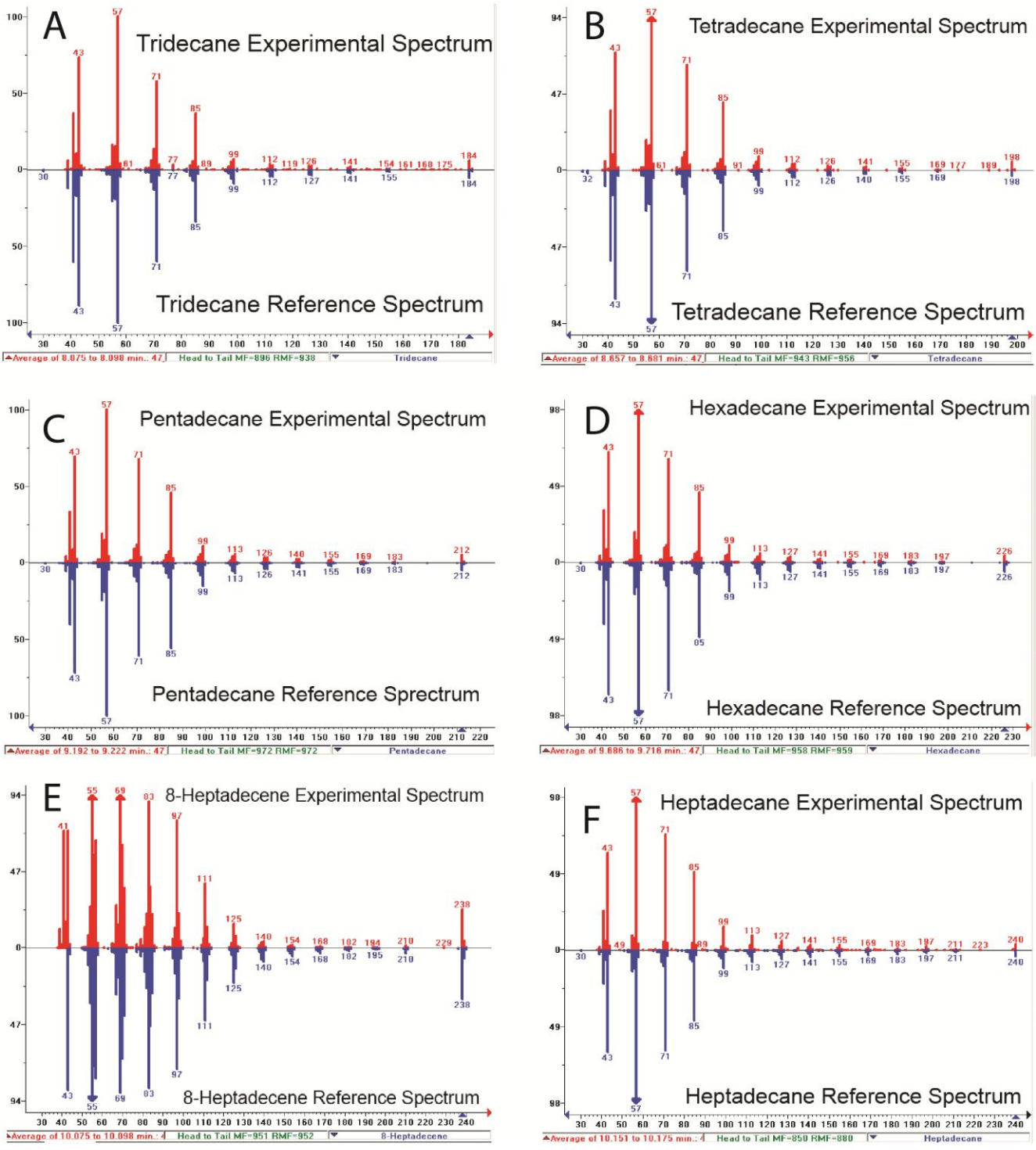
Supplemental Table 1: Alkane yield (mg/L) of *E. coli* BL21(DE3) cell cultures expressing FabH2 in the presence and absence of propanoate. Yields are reported as mean ± standard deviation of three parallel cultures. The equivalent experimental conditions (+/- FabH2, +/- IPTG, and +/- propionate) were tested in the absence of AAR and ADC, and as expected, no alkanes were detected in that experiment.



Supplemental Figure 1: FabH initiates fatty acid biosynthesis by converting acetyl-CoA into acetyl-ACP, which acts as a primer for fatty acid biosynthesis. Fatty acyl-ACPs are then converted to fatty aldehydes by Acyl-ACP reductase (AAR). These aldehydes are decarbonylated by aldehyde decarbonylase (ADC) to form an alkane that is one carbon shorter than the original acyl-ACP.

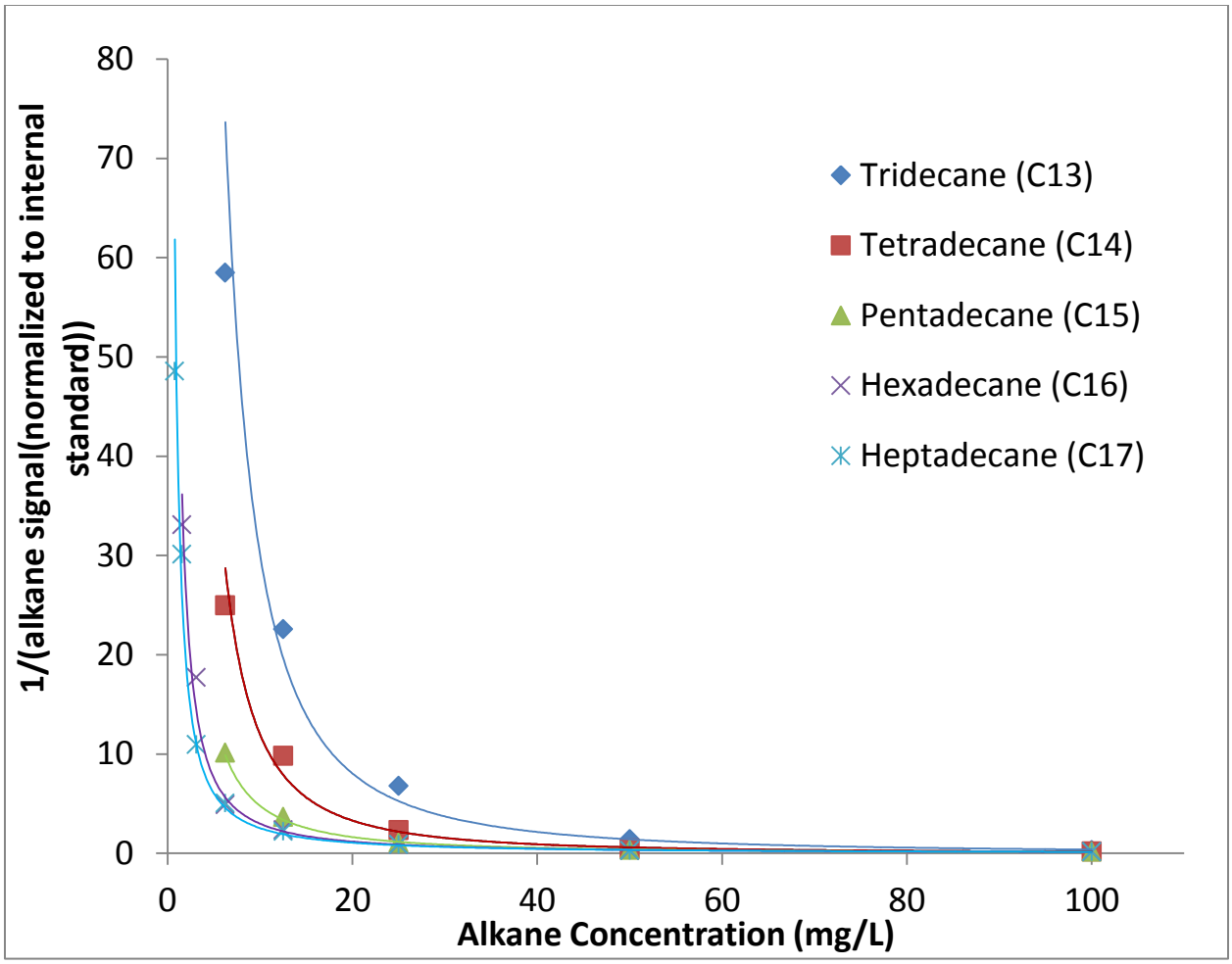


Supplemental Figure 2: Chromatograms of alkane production in *E. coli* BL21(DE3) cells expressing either AAR and ADC, FabH2, or all three enzymes as indicated. For this experiment, media did not contain propanoate. Peak identities were confirmed by spectral matching to the NIST spectral database, as well as by comparison of elution times to that of product standards.

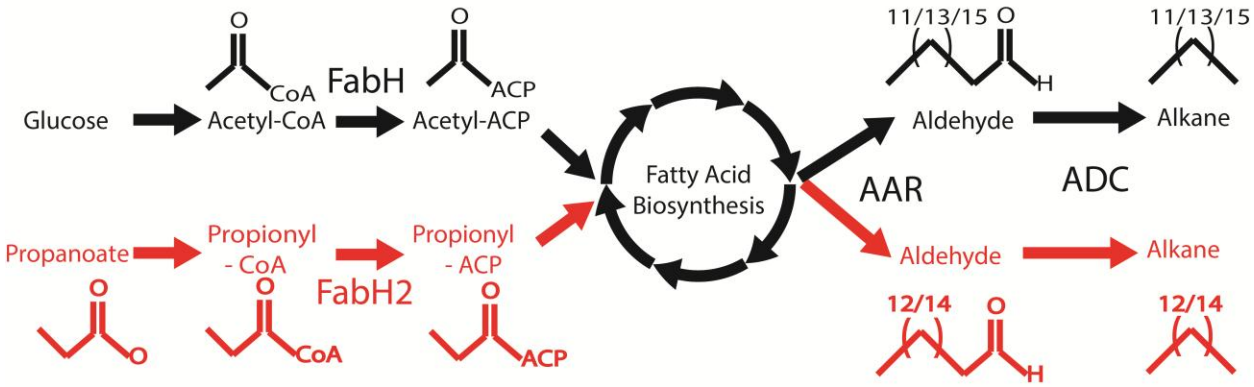


Supplemental Figure 3: Comparison of MS spectra for experimentally obtained alkanes against reference spectra for a) tridecane b) tetradecane c) pentadecane d) hexadecane e) 8-heptadecene f) heptadecane. Experimental spectra were derived from alkanes produced by *E coli* BL21(DE3) expressing the AAR and ADC pathway, and FabH2, in media supplemented with propanoate. The experimental spectra (red) were compared to the NIST reference spectral

database (blue) using NIST MS search 2.0. All identifications showed a significant R match of 880 or greater.



Supplemental Figure 4: Standard curves for alkanes in the chain length range C13-C17. All data was normalized to the internal undecane standard before analysis. All standard curves showed fit a power function with an R^2 greater than 0.99.



Supplemental Figure 5: Alkane biosynthesis in the presence of both FabH and FabH2. FabH preferentially utilizes acetyl-CoA as a priming unit, which results in the production of odd chain length alkanes (black). FabH2 demonstrates a greater preference than FabH for propionyl-CoA, thereby inducing the production of even chain length alkanes (red).