Supporting information

A multi-enzyme cascade with internal cofactor cycling for the green conversion of inert n-alkanes to α,ω-dicarboxylic acids

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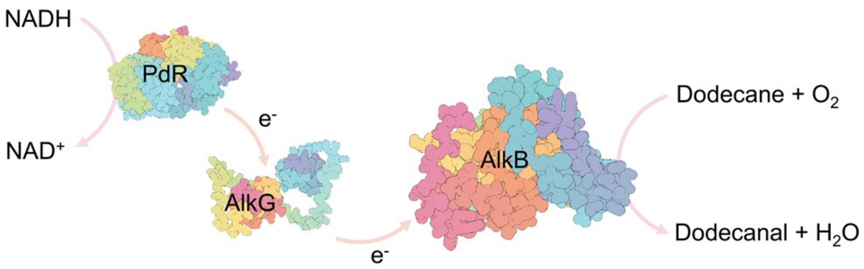
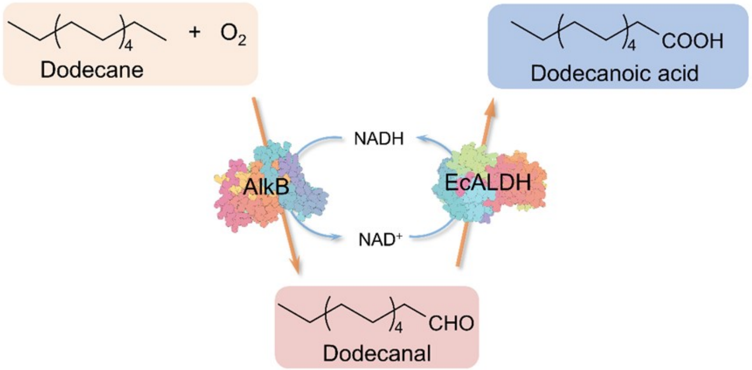
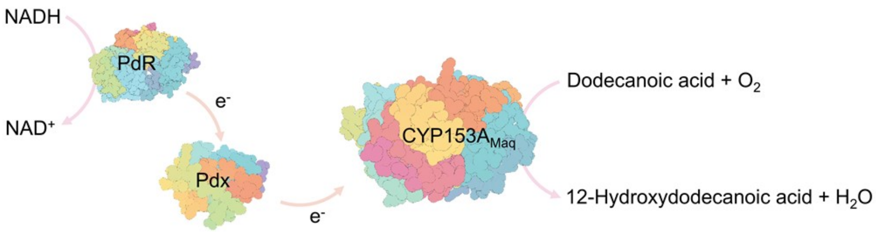


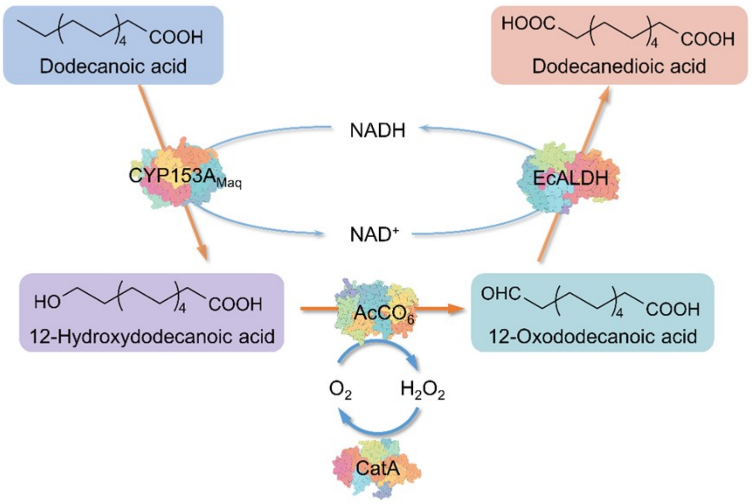
Scheme S1 The terminal C-H bond hydroxylation of dodecane catalyzed by AlkB. AlkB is a non-heme diiron monooxygenase. During the catalytic cycle, the diiron center activates molecular oxygen (O_2). One oxygen atom is inserted into the terminal C-H bond of the alkane substrate. Concurrently, the second oxygen atom acts as the terminal acceptor for the hydrogen atoms—specifically, the hydride equivalent derived from NADH (transferred via the PdR-AlkG electron cascade) and a proton—to ultimately generate a molecule of water (H_2O). Thus, the hydrogen atoms removed during this oxidation process are transferred to form H_2O.[1,2]



Scheme S2 The scheme of multi-enzymatic conversion from dodecane to dodecanoic acid coupled with NADH regeneration.



Scheme S3 ω-hydroxylation of 12-hydroxydodecanoic acid catalyzed by CYP153A$_{Maq}$. CYP153A$_{Maq}$ is a cytochrome P450 monooxygenase. In its catalytic cycle, the heme iron center activates O_2. One oxygen atom is incorporated into the ω-position of dodecanoic acid to form the hydroxyl group. The second oxygen atom serves as the terminal acceptor for the hydrogen atoms (the electron/hydride equivalents donated by NADH via the PdR-Pdx chain, along with a proton), yielding a molecule of H_2O.[3]



Scheme S4 A multi-enzyme cascade conversion scheme for the conversion of dodecanoic acid to dodecanedioic acid ingeniously integrates an internal NADH cycle regeneration system.

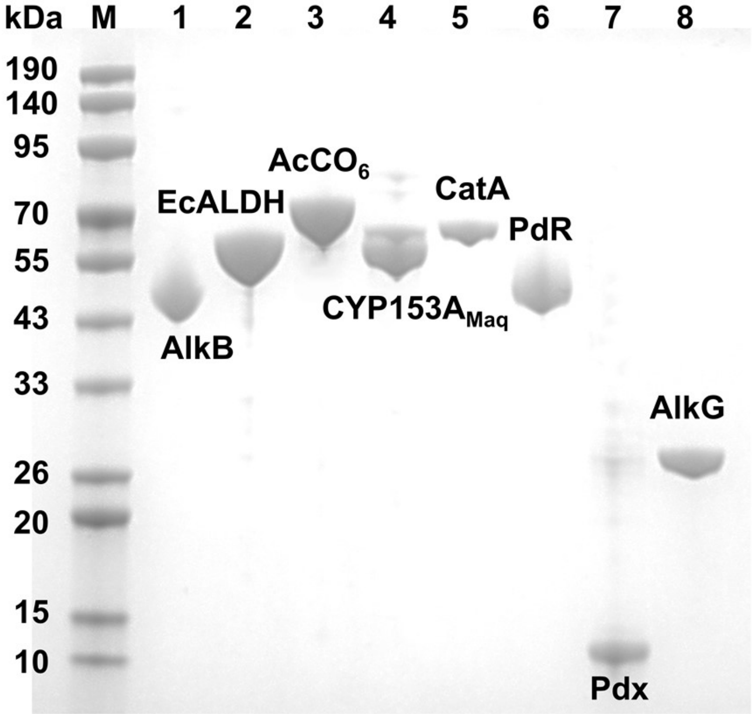


Fig. S1 SDS-PAGE analysis of purified enzymes used in this study. (a) Lane M: marker; Lane 1: AlkB (46.9 kDa); Lane 2: EcALDH (54.2 kDa); Lane 3: $AcCO_6$ (60.9 kDa); Lane 4: $CYP153A_{Maq}$ (55.1 kDa); Lane 5: CatA (55.9 kDa); Lane 6: PdR (46.6 kDa); Lane 7: Pdx (12.6 kDa); Lane 8: AlkG (20.1 kDa).

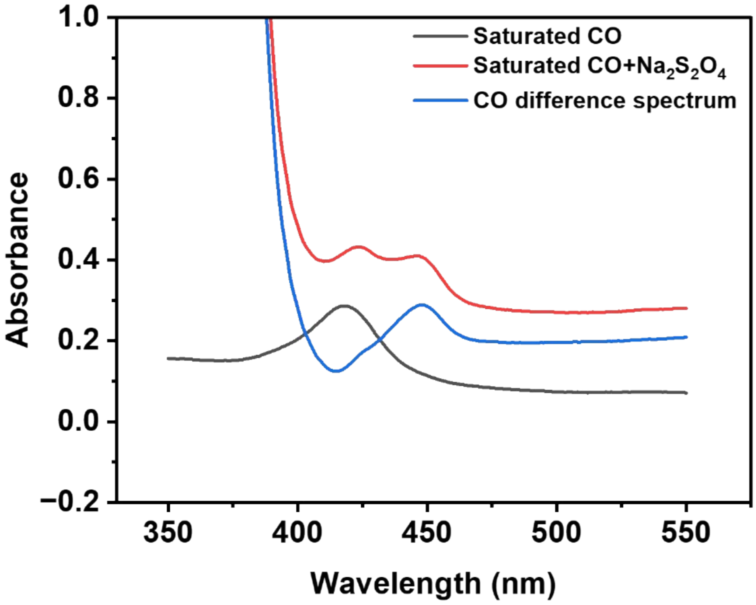


Fig. S2 CO difference spectra of purified CYP153A$_{Maq}$. Black curve: The CO statured CYP153A$_{Maq}$ sample; Red curve: Added $Na_2S_2O_4$ to the CO statured CYP153A$_{Maq}$ sample; Blue curve: CO difference spectra.

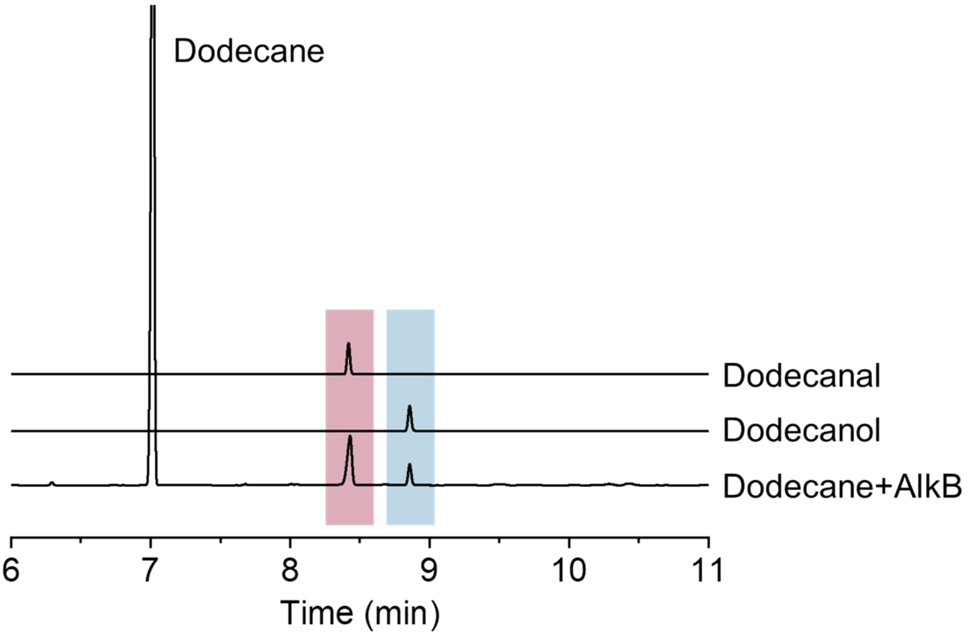


Fig. S3 GC analysis of the formation of dodecanol and dodecanal catalyzed by AlkB. The reactions were performed in a 7 mL sealed vial with magnetic string at room temperature. The 500 µL reaction mixture contained 100 mM KPi buffer (pH=8.0), 10 µM (0.06 U/mL) AlkB, 30 µM AlkG, 60 µM PdR (molar ratio of AlkB: AlkG: PdR=1:3:6), 4 mM NADH and 0.75% (*v*/*v*) dodecane as substrate. After the reactions were completed, the reaction mixtures were extracted with MTBE containing 0.5 mM n-eicosane as an internal standard.

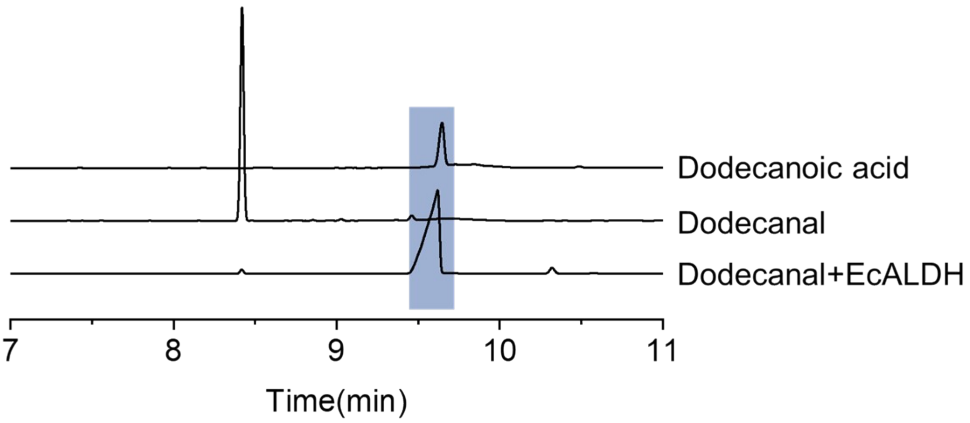


Fig. S4 GC analysis of the formation of dodecanoic acid catalyzed by EcALDH. The reactions were performed in a 7 mL sealed vial with magnetic string at room temperature. The 500 µL reaction mixture contained 100 mM KPi buffer (pH=8.0), 10 µM (0.20 U/mL) EcALDH, 4 mM NAD$^+$ and 2 mM dodecanal. After the reactions were completed, the reaction mixtures were extracted with MTBE containing 0.5 mM n-eicosane as an internal standard.

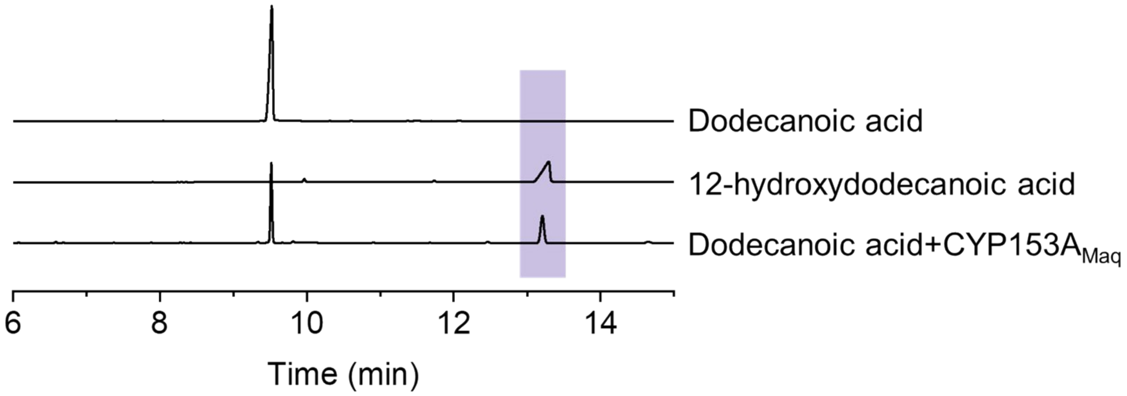


Fig. S5 GC analysis of the formation of 12-hydroxydodecanoic acid catalyzed by CYP153A$_{Maq}$. The reactions were performed in a 7 mL sealed vial with magnetic string at room temperature. The 500 µL reaction mixture contained 100 mM KPi buffer (pH=8.0), 30 mM $MgCl_2$, 10 µM (0.10 U/mL) CYP153A$_{Maq}$, 10 µM PdR, 100 µM Pdx (molar ratio of CYP153A$_{Maq}$: PdR: Pdx=1:1:10), 2 mM NADH and 2 mM dodecanoic acid. After the reaction was completed, the reaction mixture was first acidified with 10% (*v*/*v*) hydrochloric acid. Then, MTBE containing 0.5 mM n-eicosane as an internal standard was added for extraction.

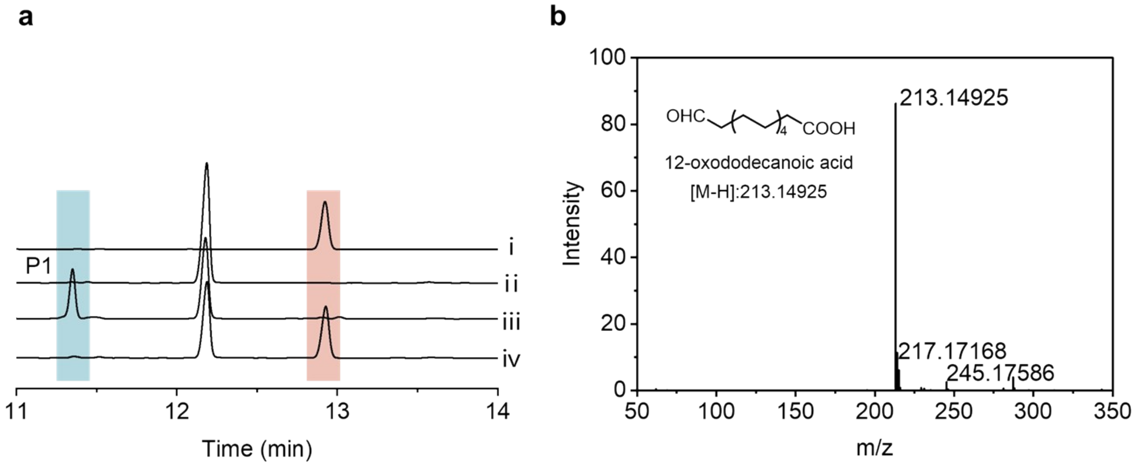


Fig. S6 a GC analysis of the formation of dodecanedioic acid catalyzed by AcCO$_6$ and EcALDH. (i) GC analysis of dodecanedioic acid standard. (ii) GC analysis of the negative control (without AcCO$_6$ and EcALDH) using 12-hydroxydodecanoic acid as the substrate. (iii) GC analysis of the conversion from 12-hydroxydodecanoic acid to 12-oxododecanoic acid catalyzed by AcCO$_6$. (iv) GC analysis of the conversion from 12-hydroxydodecanoic acid to dodecanedioic acid catalyzed by AcCO$_6$ and EcALDH. AcCO$_6$-catalyzed reactions were performed in a 7 mL sealed vial with magnetic string at room temperature. The 500 µL reaction mixture contained 100 mM KPi buffer (pH=8.0), 10 µM (0.003 U/mL) AcCO$_6$ and 2 mM 12-hydroxydodecanoic acid. Since 12-oxododecanoic acid standards were unavailable, we indirectly verified the aldehyde dehydrogenase activity of EcALDH by adding 10 µM (0.17 U/mL) EcALDH and 2 mM NAD$^+$ to the reaction mixture described above, thereby converting the intermediate 12-oxododecanoic acid into dodecanedioic acid. After the reactions were completed, the reaction mixtures were first acidified with 10% (*v*/*v*) hydrochloric acid. Then, MTBE containing 0.5 mM n-eicosane as an internal standard was added for extraction. The derivatization method reported by Slaughter et al.[4] was used for sample processing. **b** When AcCO$_6$ catalyzed the conversion of 12-hydroxydodecanoic acid, a new product peak (labeled P1) was observed (**Figure S6a**). LC-MS analysis confirmed this product to be 12-oxododecanoic acid.

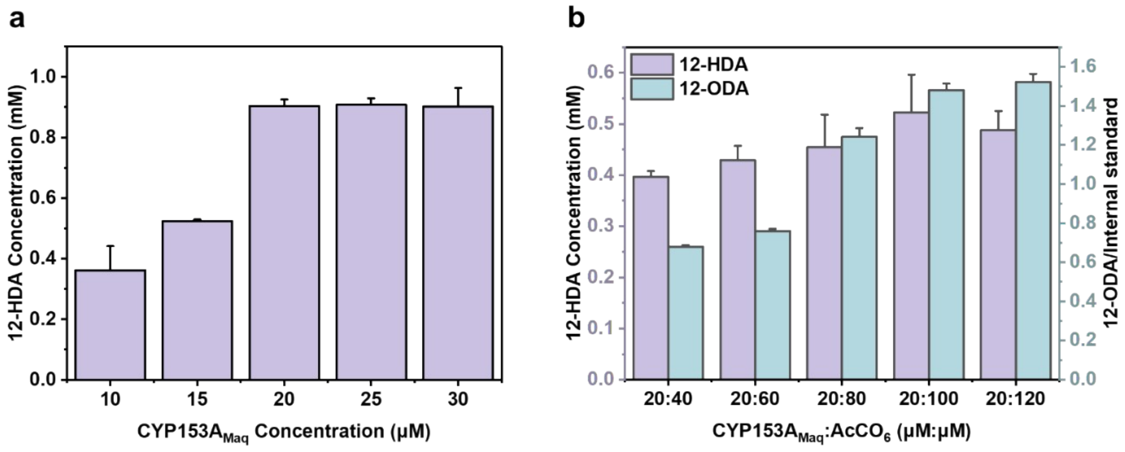


Fig. S7 Optimization of the enzyme ratio for CYP153A$_{Maq}$ to AcCO$_6$. **a** The enzyme concentration of CYP153A$_{Maq}$ was optimized. With the Mg^{2+} concentration fixed at 30 mM, CYP153A$_{Maq}$ was added at varying concentrations. **b** The enzyme ratio of CYP153A$_{Maq}$ to AcCO$_6$ was optimized. With CYP153A$_{Maq}$ concentration fixed at 20 μM (0.19 U/mL), AcCO$_6$ was added at varying concentrations.

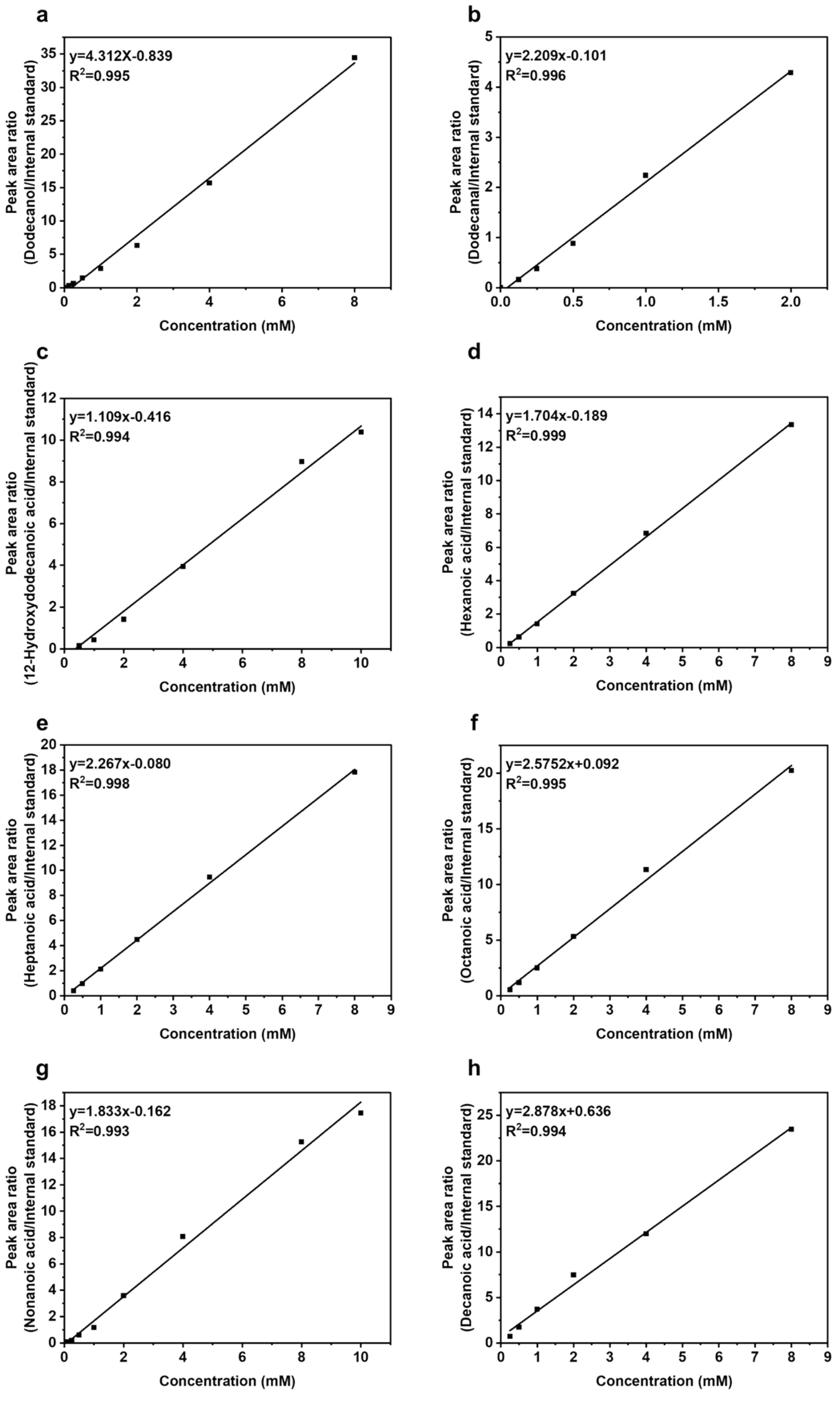


Fig. S8 GC calibration curve for the quantification analysis of dodecanol (a), dodecanal (b), 12-hydroxydodecanoic acid (c) , hexanoic acid (d), heptanoic acid (e), octanoic acid (f), nonanoic acid (g) and decanoic acid (h).

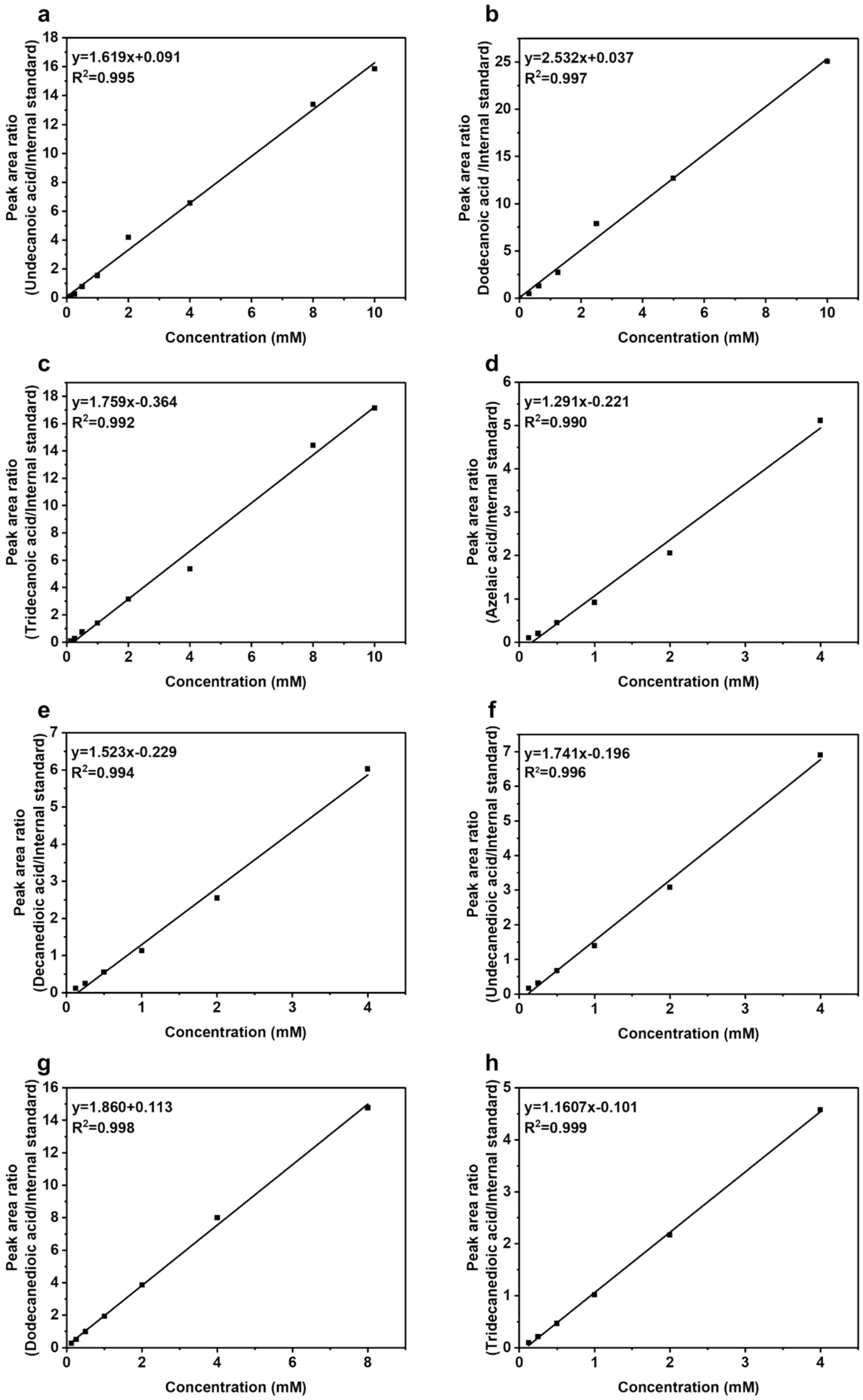


Fig. S9 GC calibration curve for the quantification analysis of undecanoic acid (a), dodecanoic acid (b), tridecanoic acid (c), azelaic acid (d), decanedioic acid (e), undecanedioic acid (f), dodecanedioic acid (g) and tridecanedioic acid (h).

Table S1. Comparison of key performance and sustainability metrics of the proposed multi-enzyme cascade system with established chemical oxidation and whole-cell fermentation methods.

Metrics	**Typical process**	**Substrate**	**Reaction conditions**	**Catalyst**	**Oxidizing agent**	**Cofactor**	**Atom economy**	**Regional selectivity**	**Ref.**
This Work	Multi-enzyme cascade reaction	Dodecane	Room temperature, room pressure	Enzymes	O_2	Internal hydrogen-borrowing cycle	All substrate atoms enter the product	100% Terminal selectivity	
Chemical synthesis	Butadiene Trimerization Route	1,3-Butadiene	High temperature (>100 °C), high pressure	Precious metals (Pd, V, $TiCl_4$)	HNO_3	Consuming stoichio-metric ratio of oxidant	Produces large amounts of N_2O	Producing short -chain heteroacids	5
Microbial fermentation	Fermentation by engineered *C. viswanathii*	Dodecane	30 °C, room pressure	Cells	O_2	Exogenous Glucose (NAD(P)H regeneration & Growth)	Fatty acid catabolism via β-oxidation	Few by-products	6
	Fermentation by engineered *Y. lipolytica*	Dodecane	28 °C, room pressure	Cells	O_2	Exogenous Glucose (NAD(P)H regeneration & Growth)	Intermediate accumulation	Few by-products	7
	Fermentation by *C. viswanathii ipe-1*	Dodecane	30 °C, room pressure	Cells	O_2	Exogenous Glucose & xylose (NAD(P)H regeneration & Growth)	Fatty acid catabolism via β-oxidation	Few by-products	8

DNA sequences and amino acid sequences of enzymes used in this stud

The AlkB gene, derived from *Pseudomonas putida* GPO1, has been codon-optimized for expression in *E. coli*. The gene sequence contains a 6×His tag, and the start and stop codons are indicated by lowercase letters and underscores.

atgCTTGAGAAACACAGAGTTCTGGATTCCGCTCCAGAGTACGTAGATAAAAAGAAATATCTCTGGATACTATCAACTTTGTG

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TACGGCGCACTTCCATTGCTTGATGCGATGTTTGGTGAGGACTTTAATAATCCGCCTGAAGAAGTGGTGCCGAAACTAGAGA

AGGAGCGGTACTATCGAGTTTTGACATATCTAACAGTTCCTATGCATTACGCTGCATTAATTGTGTCAGCATGGTGGGTCGGA

ACTCAGCCAATGTCTTGGCTTGAAATTGGTGCGCTTGCCTTGTCACTGGGTATCGTGAACGGACTAGCGCTCAATACAGGAC

ACGAACTCGGTCACAAGAAGGAGACTTTTGATCGTTGGATGGCCAAAATTGTGTTGGCTGTCGTAGGGTACGGTCACTTCTT

TATTGAGCATAATAAGGGTCATCACCGTGATGTCGCTACACCGATGGATCCTGCAACATCCCGGATGGGAGAAAGCATTTAT

AAGTTTTCAATCCGTGAGATCCCAGGAGCATTTATTCGTGCTTGGGGGCTTGAGGAACAACGCCTTTCGCGCCGTGGCCAAA

GCGTTTGGAGTTTCGATAATGAAATCCTCCAACCAATGATCATCACAGTTATTCTTTACGCCGTTCTCCTTGCCTTGTTTGGAC

CTAAGATGCTGGTGTTCCTGCCGATTCAAATGGCTTTCGGTTGGTGGCAGCTGACCAGTGCGAACTATATTGAACATTACGG

CTTGCTCCGTCAAAAAATGGAGGACGGTCGATATGAGCATCAAAAGCCGCACCATTCTTGGAATAGTAATCACATCGTCTCT

AATCTAGTGCTGTTCCACCTTCAGCGGCACTCGGATCACCACGCGCATCCAACACGTTCTTATCAGTCACTTCGGGATTTTCCC

GGCCTGCCGGCTCTTCCGACGGGTTACCCTGGTGCATTTTTGATGGCGATGATTCCTCAGTGGTTTAGATCAGTTATGGATCC

CAAGGTAGTAGATTGGGCTGGTGGTGACCTTAATAAGATCCAAATTGATGATTCGATGCGAGAAACCTATTTGAAAAAATTT

GGCACTAGTAGTGCTGGTCATAGTTCGAGTACCTCTGCGGTAGCATCGCACCACCACCACCACCACtag

MLEKHRVLDSAPEYVDKKKYLWILSTLWPATPMIGIWLANETGWGIFYGLVLLVWYGALPLLDAMFGEDFNNPPEEVVPKLEKER

YYRVLTYLTVPMHYAALIVSAWWVGTQPMSWLEIGALALSLGIVNGLALNTGHELGHKKETFDRWMAKIVLAVVGYGHFFIEHN

KGHHRDVATPMDPATSRMGESIYKFSIREIPGAFIRAWGLEEQRLSRRGQSVWSFDNEILQPMIITVILYAVLLALFGPKMLVFLPI

QMAFGWWQLTSANYIEHYGLLRQKMEDGRYEHQKPHHSWNSNHIVSNLVLFHLQRHSDHHAHPTRSYQSLRDFPGLPALPTG

YPGAFLMAMIPQWFRSVMDPKVVDWAGGDLNKIQIDDSMRETYLKKFGTSSAGHSSSTSAVASHHHHHH

The AlkG gene, derived from *Pseudomonas putida* GPO1, has been codon-optimized for expression in *E. coli*. The gene sequence has a 6×His tag, and the start and stop codons are marked with lowercase letters and underscores.

atgGCGAGCTACAAATGCCCGGATTGCAACTATGTTTATGACGAGAGCGCGGGTAATGTTCACGAAGGTTTCAGCCCGGGCACCCCGTGGCACCTGATCCCGGAAGACTGGTGCTGCCCGGATTGCGCGGTGCGTGACAAGCTGGATTTCATGCTGATTGAGAGCGGTGTGGGCGAAAAAGGTGTTACCAGCACCCACACCAGCCCGAACCTGAGCGAGGTGAGCGGCACCAGCCTGACCGCGGAAGCGGTGGTTGCGCCGACCAGCCTGGAGAAGCTGCCGAGCGCGGACGTTAAGGGTCAGGATCTGTACAAAACCCAACCGCCGCGTAGCGATGCGCAGGGTGGCAAGGCGTACCTGAAATGGATCTGCATTACCTGCGGTCACATCTATGACGAAGCGCTGGGCGATGAGGCGGAAGGTTTCACCCCGGGCACCCGTTTTGAGGACATTCCGGATGACTGGTGCTGCCCGGACTGCGGTGCGACCAAAGAAGATTATGTGCTGTATGAAGAGAAGCTCGAGCACCACCACCACCACCACtaa

MASYKCPDCNYVYDESAGNVHEGFSPGTPWHLIPEDWCCPDCAVRDKLDFMLIESGVGEKGVTSTHTSPNLSEVSGTSLTAEAVVAPTSLEKLPSADVKGQDLYKTQPPRSDAQGGKAYLKWICITCGHIYDEALGDEAEGFTPGTRFEDIPDDWCCPDCGATKEDYVLYEEKLEHHHHHH

The PdR gene, derived from *Pseudomonas putida*, has been codon-optimized for expression in *E. coli*. The gene sequence contains a 6×His tag, and the start and stop codons are indicated by lowercase letters and underscores.

atgAACGCAAACGACAACGTGGTCATCGTCGGTACCGGACTGGCTGGCGTTGAGGTCGCCTTCGGCCTGCGCGCCAGCGGCT
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GGTAACGGCTATCAACCGCGACCGACAGCAAGTAATCCTATCGGATGGCCGGGCACTGGATTACGACCGGCTGGTATTGGC
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CGAGGACGCCGAGTGCATTCGCCGGCAGCTGATTGCGGATAACCGTCTGGTGGTGATTGGTGGCGGCTACATTGGCCTTGA
AGTGGCTGCCACCGCCATCAAGGCGAACATGCACGTCACCCTGCTTGATACGGCAGCCCGGGTTCTGGAGCGGGTTACCGC
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CGAGATGTCGACCGACCAACAGAAGGTTACCGCCGTCCTCTGCGAGGACGGCACAAGGCTGCCAGCGGATCTGGTAATCGC
CGGGATTGGCCTGATACCAAACTGCGAGTTGGCCAGTGCGGCCGGCCTGCAGGTTGATAACGGCATCGTGATCAACGAACA
CATGCAGACCTCTGATCCCTTGATCATGGCCGTCGGCGACTGTGCCCGATTTCACAGTCAGCTCTATGACCGCTGGGTGCGTA
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CGCCCTGGTTCTGGTCCGATCAGTATGAGATCGGATTGAAGATGGTCGGACTGTCCGAAGGGTACGACCGGATCATTGTCC
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AGTGGAGTTCAACCAGTCAAAACAAATAATCACGGATCGTTTGCCGGTTGAACCAAACCTACTCGGTGACGAAAGCGTGCCG
TTAAAGGAAATCATCGCCGCCGCCAAAGCTGAACTGAGTAGTGCCCACCACCACCACCACCACtaa

MNANDNVVIVGTGLAGVEVAFGLRASGWEGNIRLVGDATVIPHHLPPLSKAYLAGKATAESLYLRTPDAYAAQNIQLLGGTQVTA
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GLQVDNGIVINEHMQTSDPLIMAVGDCARFHSQLYDRWVRIESVPNALEQARKIAAILCGKVPRDEAAPWFWSDQYEIGLKMV
GLSEGYDRIIVRGSLAQPDFSVFYLQGDRVLAVDTVNRPVEFNQSKQIITDRLPVEPNLLGDESVPLKEIIAAAKAELSS AHHHHHH

The EcALDH gene, derived from *Escherichia coli* K-12, has been codon-optimized for *E. coli* expression. The gene sequence contains a 6×His tag, and the start and stop codons are indicated by lowercase letters and underscores.

atgAATTTTCATCATCTGGCTTACTGGCAGGATAAAGCGTTAAGTCTCGCCATTGAAAACCGCTTATTTATTAACGGTGAATAT
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TCGTGCCGTGGAACTTCCCGCTGTTGCTGACTTGCTGGAAACTCGGCCCGGCGCTGGCGGCGGGAAACAGCGTGATTCTAA
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ACGTGGTGACGGGTTTTGGTCATGAAGCCGGGCAGGCGCTGTCGCGTCATAACGATATCGACGCCATTGCCTTTACCGGTTC
AACCCGTACCGGGAAACAGCTGCTGAAAGATGCGGGCGACAGCAACATGAAACGCGTCTGGCTGGAAGCGGGCGGCAAAA
GCGCCAACATCGTTTTCGCTGACTGCCCGGATTTGCAACAGGCGGCAAGCGCCACCGCAGCAGGCATTTTCTACAACCAGGG
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CATAGCTTTATTCGGGAAGGCGAAAGCAAAGGGCAACTGTTGTTGGATGGCCGTAACGCCGGGCTGGCTGCCGCCATCGGC
CCGACCATCTTTGTGGATGTGGACCCGAATGCGTCCTTAAGTCGCGAAGAGATTTTCGGTCCGGTGCTGGTGGTCACGCGTT
TCACATCAGAAGAACAGGCGCTACAGCTTGCCAACGACAGCCAGTACGGCCTTGGCGCGGCGGTATGGACGCGCGACCTCT
CCCGCGCGCACCGCATGAGCCGACGCCTGAAAGCCGGTTCCGTCTTCGTCAATAACTACAACGACGGCGATATGACCGTGCC
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TGGATAAGCCTGGAGGCCCACCACCACCACCACCACtaa

MNFHHLAYWQDKALSLAIENRLFINGEYTAAAENETFETVDPVTQAPLAKIARGKSVDIDRAMSAARGVFERGDWSLSSPAKRKA
VLNKLADLMEAHAEELALLETLDTGKPIRHSLRDDIPGAARAIRWYAEAIDKVYGEVATTSSHELAMIVREPVGVIAAIVPWNFPLLL
TCWKLGPALAAGNSVILKPSEKSPLSAIRLAGLAKEAGLPDGVLNVVTGFGHEAGQALSRHNDIDAIAFTGSTRTGKQLLKDAGDS
NMKRVWLEAGGKSANIVFADCPDLQQAASATAAGIFYNQGQVCIAGTRLLLEESIADEFLALLKQQAQNWQPGHPLDPATTMG
TLIDCAHADSVHSFIREGESKGQLLLDGRNAGLAAAIGPTIFVDVDPNASLSREEIFGPVLVVTRFTSEEQALQLANDSQYGLGAAV
WTRDLSRAHRMSRRLKAGSVFVNNYNDGDMTVPFGGYKQSGNGRDKSLH ALEKFTELKTIWISLEAHHHHHH

The $\text{CYP153A}_{\text{Maq}}$ gene, derived from *Marinobacter aquaeole*, has been codon-optimized for expression in *E. coli*. The gene sequence contains a 6×His tag, and the start and stop codons are indicated by lowercase letters and underscores.

atgCCAACTCTACCCAGGACATTTGATGACATCCAGAGCCGCTTGATTAACGCAACCAGCCGTGTGGTTCCGATGCAGCGCCA
AATTCAAGGGTTGAAGTTCTTGATGAGCGCGAAGCGCAAAACCTTTGGTCCGCGCCGTCCGATGCCGGAGTTCGTGGAGAC
GCCGATCCCGGATGTGAATACCTTAGCGCTGGAGGATATTGATGTGTCTAACCCGTTCCTGTATCGCCAGGGCCAATGGCGT
GCGTACTTCAAACGCTTGAGAGACGAAGCACCGGTTCACTATCAGAAAAACTCTCCGTTTGGTCCGTTTTGGTCCGTTACGCG
TTTCGAGGACATCCTGTTCGTCGATAAGAGCCATGATTTGTTTTCAGCGGAACCGCGTATTATTTTAGGTGACCCGCCTGAGG
GTCTTTCCCTGGAAATGTTTATCGCCATGGATCCACCGAAGCACGACGTTCAACGTAGCAGCGTCCAGGGTGTGGTGGCGCC
AAAGAACCTGAAGGAGATGGAAGGTCTAATCCGTTCCCGGACCGGCGACGTCCTGGACAGCTTACCGACTGATAAACCGTT
TAACTGGGTTCCGGCTGTGTCAAAAGAACTGACGGGCCGTATGCTGGCGACCCTGCTGGATTTTCCGTACGAAGAGCGTCAC
AAATTAGTAGAATGGTCGGATCGCATGGCAGGCGCAGCGAGCGCGACTGGTGGTGAGTTCGCGGACGAAAACGCCATGTT
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CGGGTTTTGACCTGATCAGCCTGCTGCAGAGCAACAAAGAAACCAAGGACCTGATAAATCGGCCTATGGAATTCATCGGCAA
CCTGACCTTGCTGATCGTTGGTGGCAATGATACCACCCGTAACTCTATGTCCGGTGGTCTGGTGGCCATGAATGAATTCCCGC
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ATGCTAGCGGTAACCGCGATGAGCGCAAGTTCGACAATCCGGACCAGTTTATTATCGATCGTAAGGACGCTCGTAACCACAT
GTCGTTCGGCTACGGTGTGCATCGTTGCATGGGCAACCGCCTTGCTGAGCTGCAACTCCGTATTCTGTGGGAGGAGATCTTG
AAGCGCTTTGATAATATTGAAGTTGTTGAGGAGCCGGAGCGCGTCCAGTCGAACTTTGTTCGTGGCTACAGCCGTCTGATGG
TTAAGTTGACGCCGAATTCCCTCGAGCACCACCACCACCACCActg

MPTLPRTFDDIQSRLINATSRVVPMQRQIQGLKFLMSAKRKTFGPRRPMPEFVETPIPDVNTLALEDIDVSNPFLYRQGQWRAYF
KRLRDEAPVHYQKNSPFGPFWSVTRFEDILFVDKSHDLFSAEPRIILGDPPEGLSLEMFIAMDPPKHDVQRSSVQGVVAPKNLKEM
EGLIRSRTGDVLDSLPTDKPFNWVPAVSKELTGRMLATLLDFPYEERHKLVEWSDRMAGAASATGGEFADENAMFDDAADMAR
SFSRLWRDKEARRAAGEEPGFDLISLLQSNKETKDLINRPMEFIGNLTLLIVGGNDTTRNSMSGGLVAMNEFPREFEKLKAKPELIP
NMVSEIIRWQTPLAYMRRIAKQDVELGGQTIKKGDRVVMWYASGNRDERKFDNPDQFIIDRKDARNHMSFGYGVHRCMGNRL
AELQLRILWEEILKRFDNIEVVEEPERVQSNFVRGYSRLMVKLTPNSLEHHHHHH

The Pdx gene, derived from *Pseudomonas putida*, has been codon-optimized for expression in *E. coli*. The gene sequence contains a 6×His tag, and the start and stop codons are indicated by lowercase letters and underscores.

atgAGTAAAGTAGTCTATGTTTCACACGATGGTACTCGTCGCGAGCTGGACGTAGCCGATGGCGTGAGCCTGATGCAAGCGGCGGTCTCCAACGGCATCTACGACATCGTGGGTGACTGTGGTGGTTCTGCGAGCTGCGCTACGTGCCACGTGTATGTTAACGAGGCGTTCACCGATAAAGTTCCGGCAGCTAATGAGCGCGAGATCGGCATGCTGGAATGCGTCACCGCAGAACTTAAGCCGAACAGCCGTCTGTGTTGCCAGATTATTATGACCCCGGAATTGGACGGCATTGTTGTTGATGTGCCAGATCGTCAGTGGCTCGAGCACCACCACCACCACCACtga

MSKVVYVSHDGTRRELDVADGVSLMQAAVSNGIYDIVGDCGGSASCATCHVYVNEAFTDKVPAANEREIGMLECVTAELKPNSRLCCQIIMTPELDGIVVDVPDRQWLEHHHHHH

The AcCO$_6$ gene, derived from *Arthrobacter cholorphenolicus*, has been codon-optimized for the *E. coli* expression system. The gene sequence contains a 9×His tag, and the start and stop codons are indicated by lowercase letters and underscores.

atgGGCAGCAGCCATCATCATCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATTGATAACATTGA
AAACCTGAGCGATCGCGGCTTTGATTATGTGGTGATTGGCGGCGGCAGCGCGGGCGCGGCGGTGGCGGCGCGCCTGAGCG
AAGATCCGGATGTGAGCGTGGCGCTGGTGGAAGCGGGCCCGGATGATCGCAACATTCCGGAAATTCTGCAGCTGGATCGCT
GGATGGAACTGCTGGAAAGCGGCTATGATTGGGATTATCCGATTGAACCGCAGGAAAACGGCAACAGCTTTATGCGCCATG
CGCGCGCGAAAGTGATGGGCGGCTGCAGCAGCCATAACGCGTGCATTGCGTTTTGGGCGCCGCGCGAAGATCTGGATGAA
TGGGAAAGCAAATATGGCGCGACCGGCTGGAACGCGGCGAACGCGTGGCCGCTGTATAAACGCCTGGAAACCAACCAGGA
TGCGGGCCCGGATGCGCCGCATCATGGCGATAGCGGCCCGGTGCATCTGATGAACGTGCCGCCGGCGGATCCGAGCGGCG
TGGCGCTGCTGGATGCGTGCGAAGAAGCGGGCATTCCGCGCGCGCGCTTTAACACCGGCACCACCGTGGTGAACGGCGCG
AACTTTTTTCAGATTAACCGCCGCGGCGATGGCACCCGCAGCAGCAGCAGCGTGAGCTATATTCATCCGATTATTGAACGCG
ATAACTTTACCCTGCTGACCGGCCTGCGCGCGCGCCAGCTGGTGTTTGATGCGGATAAACGCTGCACCGGCGTGGAAGTGG
TGGGCGGCGCGCGCGGCCGCACCCATCGCCTGACCGCGCGCCATGAAGTGATTCTGAGCACCGGCGCGATTGATAGCCCGA
AACTGCTGATGCTGAGCGGCATTGGCCCGGCGGAACATCTGGCGCAGCATGGCATTGAAGTGCTGGTGGATAGCCCGGGC
GTGGGCGAAAACCTGCAGGATCATCCGGAAGGCGTGGTGCAGTTTGAAGCGAAACAGCCGATGGTGCAGACCAGCACCCA
GTGGTGGGAAATTGGCATTTTTACCCCGACCGAAGATGGCCTGGATCGCCCGGATCTGATGATGCATTATGGCAGCACCCCG
CGCGATCGCAACACCCTGCGCCATGGCTATCCGACCACCGAAAACGGCTTTAGCCTGACCCCGAACGTGACCCATGCGCGCA
GCCGCGGCACCGTGCGCCTGCGCAGCCGCGATTTTCGCGATAAACCGATGGTGGATCCGCGCTATTTTACCGATCCGGAAG
GCCATGATATGCGCGTGATGGTGGCGGGCATTCGCAAAGCGCGCGAAATTGCGGCGCAGCCGGCGATGAGCGCGTGGACC
GGCCGCGAACTGAGCCCGGGCGTGGGCGCGCAGACCGATGAAGAACTGCAGGATTATATTCGCAAAACCCATAACACCGTG
TATCATCCGGTGGGCACCGTGCGCATGGGCGCGGATGATGATGGCATGAGCCCGCTGGATGCGCGCCTGCGCGTGAAAGG
CGTGACCGGCCTGCGCGTGGCGGATGCGAGCGTGATGCCGGAACATGTGACCGTGAACCCGAACATTACCGTGATGATGAT
TGGCGAACGCTGCGCGGATCTGATTAAAGCGGATTATGCGGGCGCGGATGCGCTGGAAGAAAAAGAACTGACCACCAGCT
TTGCGtaa

MGSSHHHHHHHHHSSGLVPRGSHIDNIENLSDRGFDYVVIGGGSAGAAVAARLSEDPDVSVALVEAGPDDRNIPEILQLDRWM
ELLESGYDWDYPIEPQENGNSFMRHARAKVMGGCSSHNACIAFWAPREDLDEWESKYGATGWNAANAWPLYKRLETNQDAG
PDAPHHGDSGPVHLMNVPPADPSGVALLDACEEAGIPRARFNTGTTVVNGANFFQINRRGDGTRSSSSVSYIHPIIERDNFTLLTG

LRARQLVFDADKRCTGVEVVGGARGRTHRLTARHEVILSTGAIDSPKLLMLSGIGPAEHLAQHGIEVLVDSPGVGENLQDHPEGV
VQFEAKQPMVQTSTQWWEIGIFTPTEDGLDRPDLMMHYGSTPRDRNTLRHGYPTTENGFSLTPNVTHARSRGTVRLRSRDFRD
KPMVDPRYFTDPEGHDMRVMVAGIRKAREIAAQPAMSAWTGRELSPGVGAQTDEELQDYIRKTHNTVYHPVGTVRMGADDD
GMSPLDARLRVKGVTGLRVADASVMPEHVTV NPNITVMMIGERCADLIKADYAGADALEEKELTTSFA

The CatA gene, derived from *Bacillus subtilis*, has been codon-optimized for the *E. coli* expression system. The gene sequence contains a 9×His tag, and the start and stop codons are indicated by lowercase letters and underscores.

atgAGCAGCAATAAACTGACCACCAGCTGGGGTGCGCCGGTTGGCGATAATCAAAATAGCATGACCGCGGGTAGCCGTGGCCCGACCCTGATTCAGGACGTGCACCTGCTGGAAAAGCTGGCGCACTTCAACCGTGAGCGTGTTCCGGAACGTGTTGTTCATGCGAAGGGTGCGGGTGCGCACGGCTATTTCGAGGTGACCAACGATGTTACCAAGTACACCAAAGCGGCGTTTCTGAGCGAAGTGGGCAAGCGTACCCCGCTGTTCATCCGTTTTAGCACCGTTGCGGGTGAGCTGGGTAGCGCGGACACCGTGCGTGATCCGCGTGGCTTCGCGGTTAAGTTTTATACCGAGGAAGGCAACTACGACATCGTGGGTAACAACACCCCGGTTTTCTTTATCCGTGACGCGATTAAATTCCCGGATTTTATTCACACCCAGAAGCGTGACCCGAAAACCCACCTGAAGAACCCGACCGCGGTGTGGGATTTTTGGAGCCTGAGCCCGGAGAGCCTGCACCAAGTTACCATCCTGATGAGCGACCGTGGTATTCCGGCGACCCTGCGTCACATGCACGGCTTCGGTAGCCACACCTTTAAGTGGACCAACGCGGAGCCGGAAGGCGTGTGGATCAAATATCACTTCAAGACCGAACAGGGTGTGAAGAACCTGGATGTTAACACCGCGGCGAAAATTGCGGGCGAGAACCCGGACTACCACACCGAGGACCTGTTCAACGCGATCGAGAACGGTGACTATCCGGCGTGGAAACTGTACGTGCAGATTATGCCGCTGGAGGATGCGAACACCTATCGTTTCGACCCGTTTGATGTGACCAAGGTTTGGAGCCAAAAAGACTACCCGCTGATCGAAGTGGGCCGTATGGTTCTGGATCGTAACCCGGAAAACTATTTCGCGGAAGTGGAACAAGCGACCTTTAGCCCGGGCACCCTGGTGCCGGGTATTGACGTTAGCCCGGATAAGATGCTGCAGGGCCGTCTGTTCGCGTATCACGACGCGCACCGTTACCGTGTGGGTGCGAACCACCAAGCGCTGCCGATCAACCGTGCGCGTAACAAAGTTAACAACTACCAGCGTGATGGTCAAATGCGTTTCGACGATAACGGTGGCGGTAGCGTGTACTATGAACCGAACAGCTTTGGCGGTCCGAAGGAGAGCCCGGAAGACAAACAGGCGGCGTATCCGGTGCAAGGCATTGCGGATAGCGTTAGCTACGACCACTATGATCACTACACCCAGGCGGGTGACCTGTACCGTCTGATGAGCGAGGATGAACGTACCCGTCTGGTGGAAAACATCGTTAACGCGATGAAACCGGTTGAGAAAGAGGAGATCAAACTGCGTCAAATTGAACACTTCTACAAAGCGGACCCGGAATACGGTAAACGTGTTGCGGAAGGTCTGGGCCTGCCGATTAAGAAGGACAGCCTCGAGCACCACCACCACCACCACtaa

MSSNKLTTSWGAPVGDNQNSMTAGSRGPTLIQDVHLLEKLAHFNRERVPERVVHAKGAGAHGYFEVTNDVTKYTKAAFLSEVGKRTPLFIRFSTVAGELGSADTVRDPRGFAVKFYTEEGNYDIVGNNTPVFFIRDAIKFPDFIHTQKRDPKTHLKNPTAVWDFWSLSPESLHQVTILMSDRGIPATLRHMHGFGSHTFKWTNAEPEGVWIKYHFKTEQGVKNLDVNTAAKIAGENPDYHTEDLFNAIENGDYPAWKLYVQIMPLEDANTYRFDPFDVTKVWSQKDYPLIEVGRMVLDRNPENYFAEVEQATFSPGTLVPGIDVSPDKMLQGRLFAYHDAHRYRVGANHQALPINRARNKVNNYQRDGQMRFDDNGGGSVYYEPNSFGGPKESPEDKQAAYPVQGIADSVSYDHYDHYTQAGDLYRLMSEDERTRLVENIVNAMKPVEKEEIKLRQIEHFYKADPEYGKRVAE GLGLPIKKDSLEHHHHHH

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