Electronic Supplementary Material (ESI) for Green Chemistry.

De novo Biosynthesis and Whole-cell Catalytic Production of Paracetamol on a Gram Scale in *Escherichia coli*

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Table S1. Strains and plasmids used in this study.

Resource	Description	Reference or source
Strains		
E. coli DH5α	For cloning work	Novagen
E. coli BL21(DE3)	For protein expression	Novagen
E. coli BL21(DE3)-pGro7	For protein expression	In this study
Escherichia coli str. K-12 substr. MG1655	For genes cloning	Novagen
PA-PABA	For the production of PABA	In this study
PA1	For the production of *p*-acetamidophenol	In this study
PA2	For the production of *p*-acetamidophenol	In this study
PA3	For the production of *p*-acetamidophenol	In this study
PAB4	For the production of *p*-acetamidophenol	In this study
PAB10	For the production of *p*-acetamidophenol	In this study
PAD4	For the production of *p*-acetamidophenol	In this study
PAE3	For the production of *p*-acetamidophenol	In this study
PADF2	For the production of *p*-acetamidophenol	In this study
plasminds		
pET-duet-1	Plasmid with ColE replication origin	Novagen
pACYC-duet-1	Plasmid with p15A replication origin	Novagen
pET-28a	Plasmid with pBR322 replication origin	Novagen
pET28a-PANAT (P3)	PANAT expressed from plasmid pET-28a	In this study
pETduet-ABH (P2)	ABH expressed from plasmid pET-Duet-1	In this study
pETduet-ABH60 (PB4)	ABH60 expressed from plasmid pET-Duet-1	In this study
pETduet-ABH47 (PB3)	Pathway constructed in pACYC-Duet-1	In this study
pETduet-ABH42 (PB2)	Pathway constructed in pACYC-Duet-1	In this study
pETduet-ABH37 (PB1)	Pathway constructed in pACYC-Duet-1	In this study
pETduet-ABH60 ^{T226V} (PB5)	For site directed mutagenesis in ABH60	In this study
pETduet-ABH60 ^{G272V}(PB6)	For site directed mutagenesis in ABH60	In this study
pETduet-ABH60 ^{G290V}(PB7)	For site directed mutagenesis in ABH60	In this study
pETduet-ABH60 $^{T226V/G272V}$(PB8)	For site directed mutagenesis in ABH60	In this study
pETduet-ABH60$^{T226V/T290V}$ (PB9)	For site directed mutagenesis in ABH60	In this study
pETduet-ABH60$^{G272V/T290V}$ (PB10)	For site directed mutagenesis in ABH60	In this study
PETduet-ABH60$^{T226V/}$ $^{G272V/T290V}$ (PB11)	For site directed mutagenesis in ABH60	In this study

pET28a-PANAT^{K211A} (PC1)	For site directed mutagenesis in PANAT	In this study
pET28a-PANAT^{K211G} (PC2)	For site directed mutagenesis in PANAT	In this study
pACYCduet-*aroF-pabAB-pabC*	Pathway constructed in pACYC-Duet-1	In this study
PF3	Pathway constructed in pET-28a	In this study
PF4	Pathway constructed in pACYC-Duet-1	In this study

Table S2. Primers for strains construction in this study.

Primers	**Sequence 5'-3'**	**Restrict enzyme sites**	**Purpose**
ABHF	TAAGGATCCGATGAGTCAGCAGG	BamHI	Used for plasmid (pETduet-ABH) construction
ABHR	TAACTCGAGCAGAACGGCGCGC	XhoI	
ABH60F	tcatcaccacagccaggatccGATGGTGCAGGGCGAAC	BamHI	Used for plasmid (pETduet-ABH60) construction
ABH60R	ggtttctttaccagactcgagTTGATGGCTAATGCTTAAAC	XhoI	
ABH47F	tcatcaccacagccaggatccGATGAGCGAAGATGGT	BamHI	Used for plasmid (pETduet-ABH47) construction
ABH47R	ggtttctttaccagactcgagCAGATGCGCGCG GCT	XhoI	
ABH42F	tcatcaccacagccaggatccGATGGCGAGCGCGACC	BamHI	Used for plasmid (pETduet-ABH42) construction
ABH42R	ggtttctttaccagactcgagCATAATGCTGCAGCC	XhoI	
ABH37F	tcatcaccacagccaggatccGATGAGCAATATTCGCG	BamHI	Used for plasmid (pETduet-ABH37) construction
ABH37R	ggtttctttaccagactcgagCAGTTTGCTCAGCAT	XhoI	
PANATF	TAACATATGGGCAGCAGCCATCAT	NdeI	Used for plasmid (pET28a-PANAT) construction
PANATR	TAACTCGAGCGCGCTAATTAAGCCCGCT	XhoI	
arofF	tcatcaccacagccaggatccGATGCAGAAAGATGCGCTGAAC	BamHI	Used for plasmid (pACYCduet-*aroF-pabAB-pabC*) construction
arofR	tttctgcatgaattcggatccCTCCTTTTACGCAACGCGCGCGGTCAG	BamHI	
pabABF	ccacagccaggatccgaattcGATGCGCGTGCTGATCATT	EcoRI	
pabABR	gcattatgcggccgcaagcttCGGAAATTCCACACCCAGC	HindIII	
pabCF	taagaaggagatatacatatgATGTTCCTGATTAACGGCCATAAAC	NdeI	
pabCR	ggtttctttaccagactcgagATTCGG	XhoI	

	ACGTTCGCATAACG		

Table S3. Sequences of the genes (*aroF*, *pabAB*, *pabC*, *PANAT*, *ABH*, *ABH60*, *ABH47*, *ABH42*, *ABH37*, *mtrF*) after codon optimization.

aroF
ATGCAGAAAGATGCGCTGAACAACGTGCATATTACCGATGAACAGGTGCTGATGACCCCGG AACAGCTGAAAGCGGCGTTTCCGCTGAGCCTGCAGCAGGAAGCGCAAATTGCGGATAGCCG CAAAAGCATTAGCGATATTATTGCGGGCCGCGATCCGCGCCTGCTGGTTGTTTGTGGACCTT GTAGCATTCATGATCCGGAAACCGCGCTGGAATATGCGCGCCGCTTTAAAGCGCTGGCGGC GGAAGTTAGCGATAGCCTGTATCTGGTGATGCGCGTGTATTTTGAAAAACCGCGCACCACC GTGGGCTGGAAAGGCTTAATTAACGATCCGCATATGGATGGCAGCTTTGATGTGGAAGCGG GCCTGCAGATTGCGCGCAAACTGCTGCTGGAACTGGTGAACATGGGCCTGCCGCTGGCGAC CGAAGCGTTAGATCCTAACAGCCCGCAATATCTGGGCGATCTGTTTAGCTGGAGCGCGATT GGCGCGCGCACCACCGAAAGCCAAACCCATCGTGAAATGGCGAGCGGCTTAAGCATGCCG GTGGGCTTTAAAAACGGCACCGATGGCAGCCTGGCGACCGCGATTAATGCGATGCGCGCGG CAGCACAACCTCATCGTTTTGTGGGCATTAATCAGGCGGGCCAGGTGGCGTTATTACAGAC CCAAGGTAATCCGGATGGTCATGTGATTCTGCGCGGCGGCAAAGCGCCGAATTATAGCCCG GCGGATGTGGCGCAATGCGAAAAAGAAATGGAACAGGCGGGCCTGCGCCCGAGCTTAATG GTTGATTGTAGCCATGGCAACAGCAACAAAGATTATCGCCGCCAGCCGGCGGTGGCGGAAA GCGTTGTTGCGCAAATTAAAGATGGCAACCGCAGCATTATTGGCCTGATGATTGAAAGCAA CATTCACGAAGGCAACCAGAGCAGCGAACAGCCGCGCAGCGAAATGAAATATGGCGTGAG CGTGACCGATGCGTGCATTAGCTGGGAAATGACCGATGCGCTGCTGCGCGAAATTCATCAG GATCTGAACGGCCAGCTGACCGCGCGCGTTGCG
pabAB
ATGCGCGTGCTGATCATTGATAACTACGACAGCTTCACCTTTAATCTGGCGACCTATGTGGA AGAAGTGACCGGTGCGGCACCAACCGTTGTTCCAAATGATGCGCAGATTGATGAAACCCTG TTTGATGCGGTGATTATTAGCCCGGGTCCAGGTCATCCAGGTGTTGCGGCGGATTTTGGTAG TTGCCGCGGCGTTATTGAACGTGGCCTGGTTCCAGTTTTGGGTGTGTGCCTGGGCCATCAAG GTATTGCGCTGGCACATGGTGGTGCAGTTGGTCCAGCACCAGTTCCAGTTCATGGTCAGGTG ACCCGCATTCATCATGATGGCAGCGAACTGTTTGATGCGATTCCGCCGCAGTTTGATGCGGT GCGCTATCATAGTCTGGTGGCGACCGATTTGCCACCGGAATTGGAAGTTACCGCGCGTACC GGCGATGGTTTGATTATGGCGCTGCGCCATCGCGAATTACCACAGTGGGGCGTGCAGTTTC ATCCGGAAAGCATTGGCGGCCAGTTTGGCCATCGCATTATGGCGAATTTTCTGAGTCTGGCG CGTCGTCAAGCACATCGTTGGGAAATTACCGAACATGTGGTGGAAACCAGCGTTGATCCGG CGGCGGTGTTTGAAACTCTGTTTGCGGGCAGCGAACATGCGTTTTGGCTGGATGATCCGCAG GGCACCACCTATATGGGTGATGCGAGCGGTCCACATGCACGTATTCGCACCCATCGTGTGG GTGAAGGCGAACTGTTTGATTGGCTGCGCGATGATCTGCGCCGTAATCGTGTTGCGCCAGGT GTTGGCTTTCGCTTAGGTTGGGTGGGCTATCTGGGCTATGAAATGAAAGCGGAATGCGGCG TGGATAATCGTCATGCGAGCAGCCATCCAGATGCGCATCTGATTTTTGCGGATCGCGCGATT GCGATTGAACCAGGCCGCGTGTGGTTAATGGCACTGGGCGAACAGGGTGAATGGTTTGCGG AAATGACTGCGGCGCTGGGTCAATTACGTCCACCACGTGCAGCGGCGGCGCCAGCAGCGCA ATTGACCGTTCGTGATGATCGCGATAGCTATCTGGATATGATTGCGCGCGCGCAGGAATTG ATTACCCGCGGCGAAAGCTATGAAATTTGCCTGACCACCCAATTGCGTGCGGAAGTGGAAG TTGATCCGTTGGCGGCGTATTTAGCTCTGCGTGCGGCGAATCCAACCAGCTATGGCAGCTTT CTGCAGTTGGGCGAAATGGCGGTTTTAAGCAGCAGCCCGGAACGCTTTATTACCATTGATG CGAGCGGTCGCGTTGAAAGCAAACCGATTAAAGGTACCCGCCCACGTGGTAGCACCGAACA

AGAAGATGCGGCGCTGATTGCGGATCTGACCGATAATCCGAAAGATCGCGCGGAAAATCTG
ATGATTGTGGATCTGGTGCGCAATGATTTAGCGCGTGGTGCGCAACCAGCAACCGTTCAAG
TGGAAAAACTGTTTGACGTGGAAACCTATGCGACCGTGCATCAGCTGGTGAGCACCATTAC
CGCGCAGCTGGAAGGCAAAGATCCGATTGATTGCGTGCGTGCGGCGTTTCCGGGTGGTAGC
ATGACTGGTGCGCCGAAAATTCGCACCATGGAGATTATTGATGAACTGGAAACCGGTCCGC
GCGGTGTTTATAGCGGTGGCCTGGGCTATTTTAGTCTGGATGGCGCGGTTGATCTGAGCATG
GTGATTCGCACCGTGGTGTATACCCCAGGCGTGCTGGAATATGGTGTGGGTGGTGCGATTTT
GGCGTTGAGTGATCCAGCGGCGGA

pabC

ATGTTCCTGATTAACGGCCATAAACAGGAAAGCCTGGCGGTTAGCGATCGTGCGACCCAAT
TTGGCGATGGCTGCTTTACCACCGCGCGCGTGATTGATGGCAAAGTGAGCCTGCTGAGCGC
GCATATTCAGCGTCTGCAGGATGCATGTCAGCGCCTGATGATTAGCTGCGATTTTTGGCCGC
AGCTGGAACAGGAAATGAAAACCCTGGCGGCGGAACAACAAAATGGCGTGCTGAAAGTGG
TTATTAGCCGCGGTAGTGGTGGTCGTGGCTATAGCACCTTGAATAGCGGTCCAGCGACCCGT
ATTTTAAGCGTGACCGCGTATCCGGCGCATTATGATCGCCTGCGCAATGAAGGTATTACCCT
GGCGCTGAGCCCAGTTCGTTTAGGCCGTAATCCACATCTGGCGGGCATTAAACATCTGAATC
GCCTGGAACAGGTGCTGATTCGCAGCCATCTGGAACAGACCAATGCGGATGAAGCGCTGGT
GCTGGATAGTGAAGGCTGGGTGACCGAATGTTGCGCGGCGAATCTGTTTTGGCGCAAAGGC
AATGTGGTGTATACCCCGCGTTTGGATCAGGCAGGCGTGAATGGTATTATGCGCCAGTTTTG
CATTCGTCTGCTGGCGCAGAGCAGTTATCAGCTGGTGGAAGTGCAAGCGAGCCTGGAAGAA
AGCCTGCAGGCGGATGAAATGGTGATTTGCAATGCGCTGATGCCGGTTATGCCGGTTTG

PANAT

ATGACTCCTCTGACCCCTGAACAGACCCATGCGTATCTGCATCATATTGGCATTGATGATCC
TGGTCCTCCGAGCCTGGCGAATTTAGATCGCCTGATCGATGCGCATTTACGCCGCGTTGCGT
TTGAAAACCTGGATGTGCTGCTGGATCGCCCGATTGAAATTGATGCGGACAAAGTGTTTGC
GAAAGTGGTGGAAGGTAGCCGTGGCGGCTATTGCTTTGAACTGAACAGCCTGTTTGCGCGT
CTGTTACTGGCGTTAGGCTATGAACTGGAACTGCTGGTGGCACGTGTTCGTTGGGGTTTACC
TGATGATGCGCCGTTAACCCAGCAGAGCCATTTAATGCTGCGCCTGTATCTGGCGGAAGGC
GAATTTCTGGTGGATGTGGGCTTTGGTTCAGCGAACCCGCCTCGTGCATTACCTTTACCGGG
CGATGAAGCAGATGCGGGTCAGGTTCATTGTGTTCGCCTGGTGGATCCTCATGCGGGCTTAT
ATGAAAGCGCGGTGCGTGGTCGTAGCGGTTGGTTACCTCTGTATCGCTTTGATCTGCGCCCG
CAGCTGTGGATTGATTATATTCCGCGCAACTGGTATACCAGCACCCATCCGCATAGCGTGTT
TCGCCAGGGTCTGAAAGCGGCGATTACCGAAGGTGATCTGCGTCTGACCTTAGCGGATGGT
CTGTTTGGTCAGCGTGCGGGTAACGGTGAAACCCTGCAGCGTCAGTTACGCGATGTGGAAG
AACTGCTGGATATTCTGCAGACCCGCTTTCGTTTACGTCTGGATCCGGCGTCAGAAGTTCCT
GCACTGGCGCGTCGTTTAGCGGGCTTAATTAGCGCGCTCGAG

ABH

ATGAGTCAGCAGGAACGTACCCGCGTGGCAATTGTGGGTGCAGGTATTGTGGGTCTGACCC
TGGCCATTGCACTGAATGCCTTTGATAAAGAACGTAAACTGGCAATTGATATCTATGAAAA
TGCAAGCGAACTGGCAGAAATTGGTGCCGGCATTAATGTTTGGCCGCGCACCCTGGCAATT
TTTAAACAGATTGGTGTGGAAGATGCCCTGATTCCGCTGCTGGATCATATTCCGGATCTGGA
ACCGCGTATTATTTTTGGTATTCGTAAAGGTGACGAAAAGAATGGCTATCAGGTTTATGATA
CCATGAATAATGGCGGTGCACTGCGCGTTCATCGTGCCCATCTGCAGAATACCCTGATTCAG
CATCTGCCGCTGCCGGGCAGTAAAGTGACCGAAATTAATAGCATTTGTGGCTTTCATCTGGG
CCATAATCTGATTGATTATAGTCATCATAGCAGCAGTGGCCAGGGTCCGCTGACCCTGCATT
TTAGCGATGGCAAACCGAGTCGCACCTGTGATATTCTGGTGGGCGCAGATGGCATTAAGAG

TACCCTGCGTCATCTGTTTCTGCCGCGCCTGCCGAATCCGGAAAAATATCTGAATTGCTATG
AACCGAAATGGAAAGGTCTGCTGGCATATCGTGGTCTGGTTCCGAAAGAAAAACTGGAAGC
CGTGAGCCCGGGCCATCGTGCCCTGACACATCCGGGCCTGATGTATAGCGGTAAAAGTGCC
TATGCCGTGGTGTATCCGGTGAGTAATGGTAAATTCATTAATGTTGTGGCCATTGTGCATGA
TAATCCGACCAATAGCACCGTGTGGCCGGGTCCGTGGCGTATGGATGTGACCCAGAGTGAA
TTTTTCGAAGTTTATAAAGGTTGGGATGAAGAAGTTCTGGATCTGATTCGCTGTGTGGATAA
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TTCTGATGGGCGATGCCGCACATGCAATGCTGCCGCATCTGGGTGCAGGCGCCCATGTTGGT
ATGGAAGATGCCTATATTCTGGCCAGTCTGATTACCCATAGTAGTACCCCGATTTGGCCGAG
CACCCAGCATGTGAGTGAAATTGCCAATATCTATAATACCATGCGCATTCCGCGCGCAGTTA
GCATGAGTAATAGCACCGATGAAGCAGGTTATCTGTGTAATCTGGAAAATCCGGGTCTGGA
AGAATTCAAAGTTGGCGATCATATTCCTAAAGAACTGCTGATTCAGACCGCCCGTACCATG
GAAAAGAAATGGGCATGGACCACCACCTATGCCGATGAAGATCGCATTAAGGCAATTAGTC
TGCTGGAAGGTCCGCGCGCCGTTCTG

ABH60

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TGGCCATTGCACTGAATGCCTTTGATAAAGAACGTAAACTGGCAATTGATATCTATGAAAA
TGCAAGCGAACTGGCAGAAATTGGTGCCGGCATTAATGTTTGGCCGCGCACCCTGGCAATT
TTTAAACAGATTGGTGTGGAAGATGCCCTGATTCCGCTGCTGGATCATATTCCGGATCTGGA
ACCGCGTATTATTTTTGGTATTCGTAAAGGTGACGAAAAGAATGGCTATCAGGTTTATGATA
CCATGAATAATGGCGGTGCACTGCGCGTTCATCGTGCCCATCTGCAGAATACCCTGATTCAG
CATCTGCCGCTGCCGGGCAGTAAAGTGACCGAAATTAATAGCATTTGTGGCTTTCATCTGGG
CCATAATCTGATTGATTATAGTCATCATAGCAGCAGTGGCCAGGGTCCGCTGACCCTGCATT
TTAGCGATGGCAAACCGAGTCGCACCTGTGATATTCTGGTGGGCGCAGATGGCATTAAGAG
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AACCGAAATGGAAAGGTCTGCTGGCATATCGTGGTCTGGTTCCGAAAGAAAAACTGGAAGC
CGTGAGCCCGGGCCATCGTGCCCTGACACATCCGGGCCTGATGTATAGCGGTAAAAGTGCC
TATGCCGTGGTGTATCCGGTGAGTAATGGTAAATTCATTAATGTTGTGGCCATTGTGCATGA
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ATGGAAGATGCCTATATTCTGGCCAGTCTGATTACCCATAGTAGTACCCCGATTTGGCCGAG
CACCCAGCATGTGAGTGAAATTGCCAATATCTATAATACCATGCGCATTCCGCGCGCAGTTA
GCATGAGTAATAGCACCGATGAAGCAGGTTATCTGTGTAATCTGGAAAATCCGGGTCTGGA
AGAATTCAAAGTTGGCGATCATATTCCTAAAGAACTGCTGATTCAGACCGCCCGTACCATG
GAAAAGAAATGGGCATGGACCACCACCTATGCCGATGAAGATCGCATTAAGGCAATTAGTC
TGCTGGAAGGTCCGCGCGCCGTTCTG

ABH47

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TGGCGGTGACCCTGAATGCGTTTGATACCGAACAGAAATTTGTGATCGATATGTACGAGAG
CGCGCCGGAACTGAGCGAAATTGGCGCGGGTATTAATGTGTGGCCGCGCACCTGGCAAATT
CTGAAAGAAATTGGCATGGAAGAGACCCTGGAACCGCTGTTTGATCATCGCCCGGATCTGG
AACGCCGCGTGGTTTTTGAAGCGCGCAAAGCGGATCAGCGCACCGGCTTTAAAGTGGTGGA
TGTGATGAAAGATGGCGGCGTGCTGCGCATTCATCGCGCGGATTTTCAGCGCAGCCTGCTG
AAACATCTGCCGGTGGCGGGTAGCAGCACCAAAATTAATACCACCTGCACCCTGCATCTGA

GCCATCGCTTAGTGGATTATGGCCCGACCCCAAGCAGCAGCAGCGCGCAACATAGCGGTCC
GGTTACCTTATATTTTACCGATAAACCGTGCGCGATTATTGACCATCTGATTCTGGTGCCGT
GCATTCGCCAGCCGACCAAATGGCCATTGCAACGTTTGAATCATCTGGATCTGTATGCGGA
AGGCCGCGTGATTCTGATTGGCGATGCGGCGCATGCGATGGTGCCACATCAAGGCGCGGGT
GCGGGTGTTGGTATTGAATATATGGGTAAAGAAAAGCACGCGGTGGTGTATCCGATTAGCG
GCGGCAAACTGATTAATATTGTGGCGACCGTGCATGATCGCAGCAAAGAAGGCACCGTGTA
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GAAGAAGAATTTCAGGCGCTGCTGCGCTGCATTCGCCAGCCAACTAAATGGCCGCTGCAAC
GCTTGAATCATCTGGATCTGTATGCGGAAGGCCGCGTGATTCTGATTGGCGATGCGGCGCAT
GCGATGGTGCCACATCAAGGTGCGGGTGCGGGTGTTGGTATTGAAGATGCGTATATTCTGG
CGAGCCTGCTGACCCAGACCAGCGAAAGCCATCCGTTACCAATGCAGCGCATTAGCCATCT
GGTGGATGTGTATAATACCGTGCGCGTGCCGCTGGCGACCAGCTTTGCGAAAGCGAGCATT
AATCAGGGCCGCTATTATGGCCTGGAAGCGCCGGGTTTTGAACATATTAAAGAAGGCGATG
ATGTGCCGCGCGATAAACTGACCGCGCTGTTACGCTTAGCGGATGATAATTGGAGCTGGAC
CACCAGCAATCCGGAAAGCGATAAAAAACGCGCGATGGAACTGCTGCAGGAAAGCCGCGC
GCATCTG

ABH42

ATGGCGAGCGCGACCCAAAAACCGTTGCGTGTTGCGATTGTGGGCGCGGGCATTGGTGGTT
TAATGTTGACCATTGCGCTGCAGCATATGGATAAAGCGAAAAAACTGGATATCCACGTGTA
CGAGGCGGCGCCGGCACTGGGTGAAATTGGTGCAGGTATTAATCTGTGGCTGCGCAGCTGG
GAAATTATGAAAGCGATTGGCCTGGAAGAAACCTTTGTGAAACTGATGGATGAAGCGCCGA
CCGATGAAAGCCGCCTGGTTTTTCAACTGCGCAAAAGCGATCAGCGCGAAGGCGAAGCGTT
AAGCGATCTGATGATGAAAGGCGGCACCATGCGCTTTCATCGCGCGGCGTTACAAAAAGCG
CTGCTGGCACGTGTTGGTGATTGTGTTAGCCTGAGCCGCCGCTTGGTTACCTATGCGGAATA
TGAAAATCATGTGGAGCTGGAATTTCAGGACGGCTTTATTGTGAATTGCGATGTGCTGATTG
CGGCGGATGGCATTAAAAGCGTGGTGCGCAAACAGTTTATGAAAAGCCTGGCGAAAGGCA
CCTATATTGTGAATAGCGATCCGGTGTGGACCGGCACCTATGCGTATCGCGGTTTGTTGAGC
CATGAAGCGATTGAACGCGAATTTCCGGGCCATCGCGCGACCAAAAATGCGGTGATTTATT
GCGGCAAATATAAGCACCTGGTGGTGTATCCGCTGAGCAATGGCCGCGATACCAATGTGGT
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CGCGAGTCGTAAAGATATTCCGCGTATTGCGGAAGTGTATAATAAAATTCGCTGCCCGGCG
GCGAATGCGATTCTGATGGCGAGCCGTGATCAGGGCAAATGGTGTGAACTGGATCATCCGG
AACTGGAAGGCGTGAATGAAGGCGATATTCTGCCGAAAGAACGCCTGGAAGGCCTGTGCC
GTGATATTAGCCAAGCGTGGGAATGGGTGTGGAAAACCAGCGCGGAAACCGATCGCCGCG
AAGCGATTCGTATGCTGGGCGAAAAAGAAGTGGGCGGCTGCAGCATTATG

ABH37

ATGAGCAATATTCGCGTGGCGGTGATTGGCGGCGGCATTGGTGGTTTAACCTTTTGCGTTGC
GCTGGGCAAACAGAGCAATATTAAAGTGGATATGTATGAGGCGGCGCGCGAATTTGGCGA
AGTGGGCGCGGGTATTGCGATGTGGTATCGCACTCAACAGATTATGGAAAAACTGGGCCTG
CATAATGATCTGGAAGCGCTGGCGGGCGGCGCGAAAAAAGGTGCGGCGGAATTAGCGTTTC
ATTTTCGCAAAAGCGATCAGCCGGAAGGCCTGAGCTTTTATGATATGAATACCCGCGGCGG
CATGTGGGCGTTTCATCGCGCAAAATTTCAGAAAACCATTATCAACCACCTGCCGCCGACCT

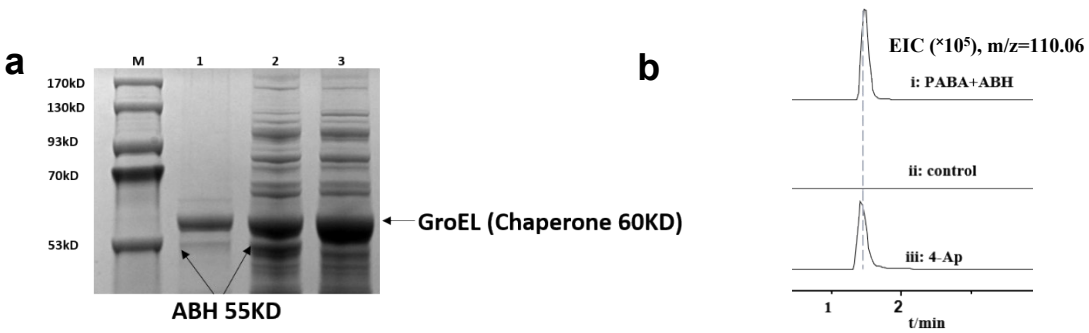
GCACCAGCCATTTTGGTAAACGCCTGGTGGCGTATGATGCGCCGGTTAGCGGTCCAATTACC CTGCATTTTAAAGATGGCACCGCGGCGGAATGCGATGTGCTGGTTGGTGCGGATGGCATTA AAAGCACCGTGCGCGCGATTATGTATAGCAATCTGGTGAAAGAAGGCAAGATTACCAAAG AGGAAGTGCTGGAACCGAATCCGGTGTGGAGCGGCACCGTTGCGTATCGTGGTTTAATTAG CAAAGAACGCCTGGAAGCGAAAATTCTGGGCCATCGCGCGCTGACCCGTGCGGTTTTGCAT ATGGGTAAAAATAAACACCTGATCATCTTCCCGATCAACGACGCGCTGATTAACGTGGTGG CGTTTTGCAGCGATTTTAGCAAAGAAGGCACCCAGTATAGCGAAGATAGCGGCGATTGGGT GGTGGATGTGCCGAAAGAAGAACTGCTGCAGCAGTATGAAGGCTGGGAACCGGAAGTGAT GGCGGTGTTGGAATGCATTGAAAAACCGAGCAAATGGGCGATTAATGTGGTGCGCCCGCCG AGCACCTATGTGAGTGGTCATGTTGCGATTCTGGGCGATAGCGCGCATGCGATGACCCCGC ATTTAGGCAGTGGTGCGGGTCAAGCGATTGAAGATGCGTATATTCTGGCGGCGCTGCTGGC GAATCCGAAATGCACTTTAGCGAGCTTACCGCGCGTGTTGCAGATTTATGATGAAGTGCGC CGCCCGAAAGCGAATAATGTGTGGAATCTGAGCCGCAAAAATGGCAGCATGTATGAATTTG CGGGCCCGGTGTGCGGCGAATTTCGCTTACATGATGATAATTTTAATAGCGAGAGCCTGGC GAAAATCGCGAAAGTGGTGATGGAAAATTATGAGTGGGCGTGGAATACCAGCGCGGAAGA AGATCGCAATCAGGCGGTGAGCATGCTGAGCAAACTG
mtrF
ATGAGCCAGACCGATGCGCGTCGTAGCGGTCGTTTTCTGCGTACCGTTGAATGGCTGGGCA ACATGCTGCCGCATCCGGTGACGTTATTTATTATTTTTATTGTGCTGCTGCTGATCGCGAGCG CGGTGGGCGCGTATTTTGGCCTGAGCGTTCCTGATCCGCGCCCGGTTGGTGCAAAAGGTCGC GCGGATGATGGCTTAATTCATGTGGTGAGCCTGCTGGATGCGGATGGCCTGATTAAAATTCT GACCCATACCGTGAAAAACTTCACCGGCTTTGCGCCGCTGGGCACCGTTTTAGTGAGCCTGT TAGGCGTGGGTATTGCGGAGAAAAGCGGCCTGATTAGCGCGTTAATGCGCCTGTTATTAAC CAAAAGCCCGCGCAAACTGACCACCTTTATGGTGGTGTTTACCGGCATTCTGAGCAACACC GCGAGCGAACTGGGCTATGTGGTGCTGATTCCGCTGAGCGCGGTGATTTTTCATAGCCTGGG CCGCCATCCGCTGGCGGGTTTAGCGGCGGCGTTTGCGGGTGTTAGCGGTGGTTATAGCGCG AATCTGTTTCTGGGCACCATTGATCCGCTGCTGGCGGGCATTACCCAGCAGGCGGCGCAAA TTATTCATCCGGATTATGTGGTGGGCCCGGAAGCGAACTGGTTCTTTATGGCGGCGAGCACC TTTGTGATTGCGCTGATTGGCTATTTTGTGACCGAAAAGATTGTGGAGCCGCAGCTGGGCCC GTATCAGAGCGATTTAAGCCAGGAAGAAAAAGATATTCGCCATAGCAACGAAATTACCCCG CTGGAATATAAAGGCCTGATTTGGGCGGGCGTGGTGTTTATTGCGCTGAGCGCGTTACTGGC GTGGAGCATTGTGCCGGCGGATGGCATTCTGCGCCATCCGGAAACCGGCCTGGTTGCGGGT TCACCATTTTTAAAAAGCATTGTGGTGTTTATCTTCCTGCTGTTTGCGCTGCCGGGCATTGTG TATGGCCGCATTACCCGCAGCCTGCGCGGTGAACGTGAAGTTGTTAACGCGATGGCGGAAA GCATGAGCACCCTGGGCTTATATCTGGTGATTATCTTCTTTGCGGCGCAGTTTGTGGCGTTCT TTAACTGGACCAACATTGGCCAGTATATTGCGGTGAAAGGCGCGGTGTTTCTGAAAGAAGT GGGCCTGGCGGGCAGCGTGCTGTTTATTGGCTTTATTCTGATTTGCGCGTTTATCAACCTGA TGATTGGCAGCGCGTCGGCGCAGTGGGCCGTTACCGCGCCTATTTTTGTTCCGATGCTGATG CTGGCGGGCTATGCGCCGGAAGTGATTCAGGCGGCATATCGCATTGGCGATAGCGTGACCA ACATTATTACCCCGATGATGAGCTATTTTGGCCTGATTATGGCGACCGTGATGAAATATAAA AAGGACGCGGGCGTGGGCACCCTGATTAGCATGATGCTGCCGTATAGCGCGTTTTTCCTGAT TGCGTGGATTGCGCTGTTTTGCATTTGGGTGTTTGTGCTGGGCCTGCCGGTGGGCCCTGGTG CGCCTACCTTGTATCCTGCGCCTTAA

Table S4. Primers for point mutation (ABH and PANAT) in this study.

primers	Sequence 5'-3'	Purpose

ABH60226F	TTGGAAGAAGTGTATCCGGG	Used for plasmid (pETduet-ABH60 $_{T226V}$) construction
ABH60226R	CGATGGCCCGGATACACTTC	
ABH60272F	CCAGCAATGATACCGTGGTG	Used for plasmid (pETduet-ABH60 $_{T272V}$) construction
ABH60272R	GCCTTTCCACACCACGGTAT	
ABH60290F	TTTTTTCACGTGTATCAGGGC	Used for plasmid (pETduet-ABH60 $_{T290V}$) construction
ABH60290R	TCAAAGCCCTGATACACGTG	
PANAT211AF	GCGGCGGCGATTACCGAAG	Used for plasmid (pET28a-PANAT$_{K211A}$) construction
PANAT211AR	ATCCGGATATAGTTCCTCCTTTC	
PANAT211CF	CAGGGTCTGTGTGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211C}$) construction
PANAT211CR	GTAATCGCCGCACACAGACC	
PANAT211GF	CAGGGTCTGGGCGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211G}$) construction
PANAT211GR	TAATCGCCGCGCCCAGACC	
PANAT211DF	AGGGTCTGGATGCGGCGATT	Used for plasmid (pET28a-PANAT$_{K211D}$) construction
PANAT211DR	GGTAATCGCCGCATCCAGAC	
PANAT211SF	AGGGTCTGAGCGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211S}$) construction
PANAT211SR	GTAATCGCCGCGCTCAGAC	
PANAT211QF	CAGGGTCTGCAGGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211Q}$) construction
PANAT211QR	GGTAATCGCCGCCTGCAGAC	
PANAT211VF	CAGGGTCTGGTTGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211V}$) construction
PANAT211VR	GGTAATCGCCGCAACCAGAC	
PANAT211WF	GGGTCTGTGGGCGGCGATT	Used for plasmid (pET28a-PANAT$_{K211W}$) construction
PANAT211WR	TCGGTAATCGCCGCCCACAG	
PANAT211HF	AGGGTCTGCATGCGGCGATT	Used for plasmid (pET28a-PANAT$_{K211H}$) construction
PANAT211HR	CGGTAATCGCCGCATGCAG	
PANAT211RF	GGTCTGCGTGCGGCGATTAC	Used for plasmid (pET28a-PANAT$_{K211R}$) construction
PANAT211RR	CGGTAATCGCCGCACGCAG	
PANAT211PF	AGGGTCTGCCTGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211P}$) construction
PANAT211PR	GGTAATCGCCGCAGGCAGAC	
PANAT211LF	AGGGTCTGTTAGCGGCGATT	Used for plasmid (pET28a-PANAT$_{K211L}$) construction
PANAT211LR	GGTAATCGCCGCTAACAGAC	
PANAT211IF	CAGGGTCTGTTTGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211I}$) construction
PANAT211IR	GGTAATCGCCGCAAACAGAC	
PANAT211TF	CAGGGTCTGACTGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211T}$) construction
PANAT211TR	GTAATCGCCGCAGTCAGAC	
PANAT211YF	CAGGGTCTGTATGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211Y}$)

PANAT211YR	GGTAATCGCCGCATACAGAC	construction
PANAT211MF	CAGGGTCTGATGGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211M}$) construction
PANAT211MR	GGTAATCGCCGCCATCAGAC	
PANAT211NF	CAGGGTCTGAATGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211N}$) construction
PANAT211NR	GGTAATCGCCGCATTCAGAC	
PANAT211FF	CAGGGTCTGTTTGCGGCGAT	Used for plasmid (pET28a-PANAT$_{K211F}$) construction
PANAT211FR	GGTAATCGCCGCAAACAGAC	



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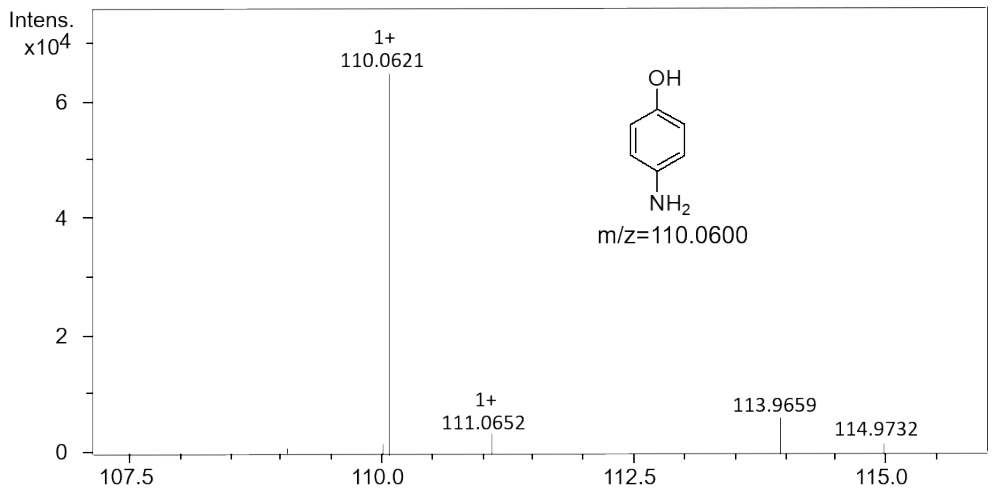
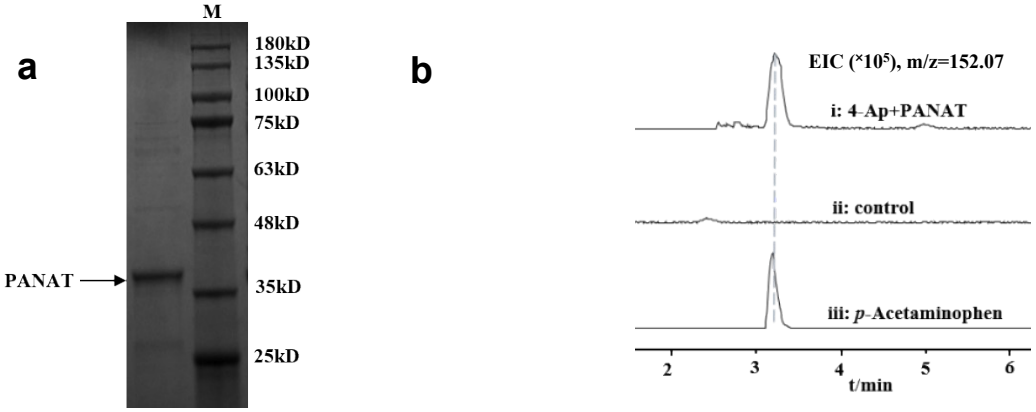


Figure S1. *In vitro* characterization of ABH. a) SDS-PAGE of isolated ABH and whole cell. The size of protein ABH and chaperone GroEL are about 55 kD and 60 kD, respectively. M: marker (solarbio. PR1910, MOPS buffer); line1: purified target protein (300 mM imidazole elution from Ni-NTA); line2: *E. coli* BL21 (DE3)-pGro7-ABH whole-cell; line3: control, *E. coli* BL21 (DE3)-pGro7 whole-cell. Criterion MOPS gel (12% precast, Biorad) was used. b) LC-MS analysis of the reaction mixtures of PABA and ABH. I: PABA (**3**) and ABH; ii: PABA (**3**); iii: 4-Ap (**2**) standard. c) Characterization of 4-Ap by ESI-MS.



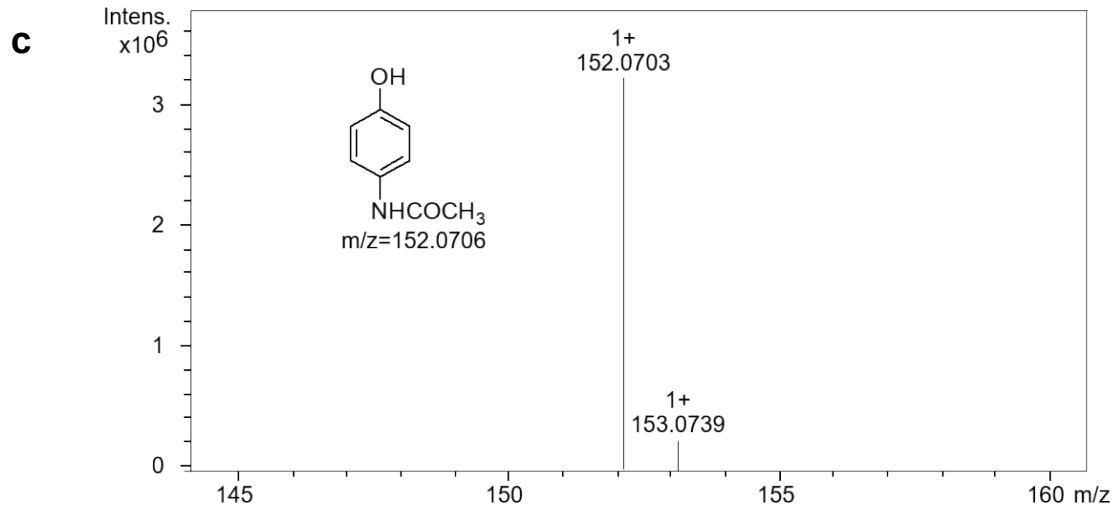


Figure S2. *In vitro* characterization of PANAT. a) SDS-PAGE of purified PANAT (about 37 kD). Criterion MOPS gel (12% precast, Biorad) was used. b) LC-MS analysis of the reaction mixtures of 4-Ap and PANAT. I: 4-Ap (**2**) and PANAT; ii: 4-Ap (**2**); iii: *p*-Acetaminophen (APAP, **1**) standard. c) Characterization of APAP by ESI-MS.

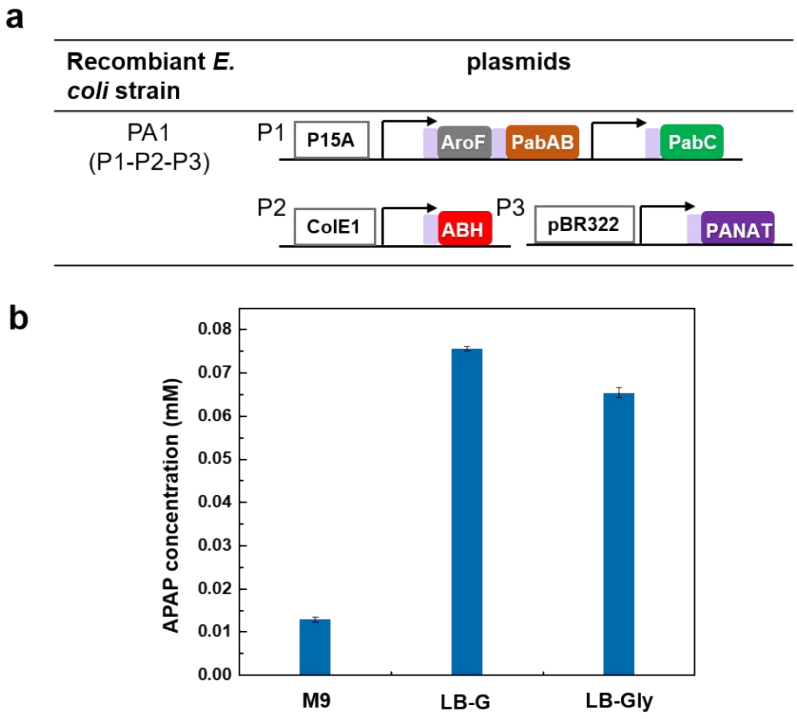


Figure S3. *In vivo* production of APAP (1) in *E. coli* PA1 using the plasmids P1-P2-P3, measured by HPLC after 48h. a) Construction of *E. coli* cells expressing AroF; PabAB; PabC; PANAT; ABH. Gray filled rectangle: AroF gene; brown filled rectangle: PabAB gene; green filled rectangle: PabC gene; purple filled rectangle: PANAT gene; red filled rectangle: ABH; light purple rectangle: ribosome binding site (RBS). b) Engineered *E. coli* cells for the production of APAP in different mediums (M9, LB-G, and LB-Gly, respectively). P15A: pACYC-Duet-1; pBR322: pET-28a; ColE1: pET-Duet-1; arrow: T7 promoter. Proteins: AroF, DAHP synthetase feedback inhibited by tyrosine; PabAB, *p*-aminobenzoic acid synthase; PabC, 4ADC lyase; ABH, 4-aminobenzoate hydroxylase; PANAT, arylamine N-acetyltransferase. The data shown in b are from three experiment replicates and are expressed as the mean value ± SD.

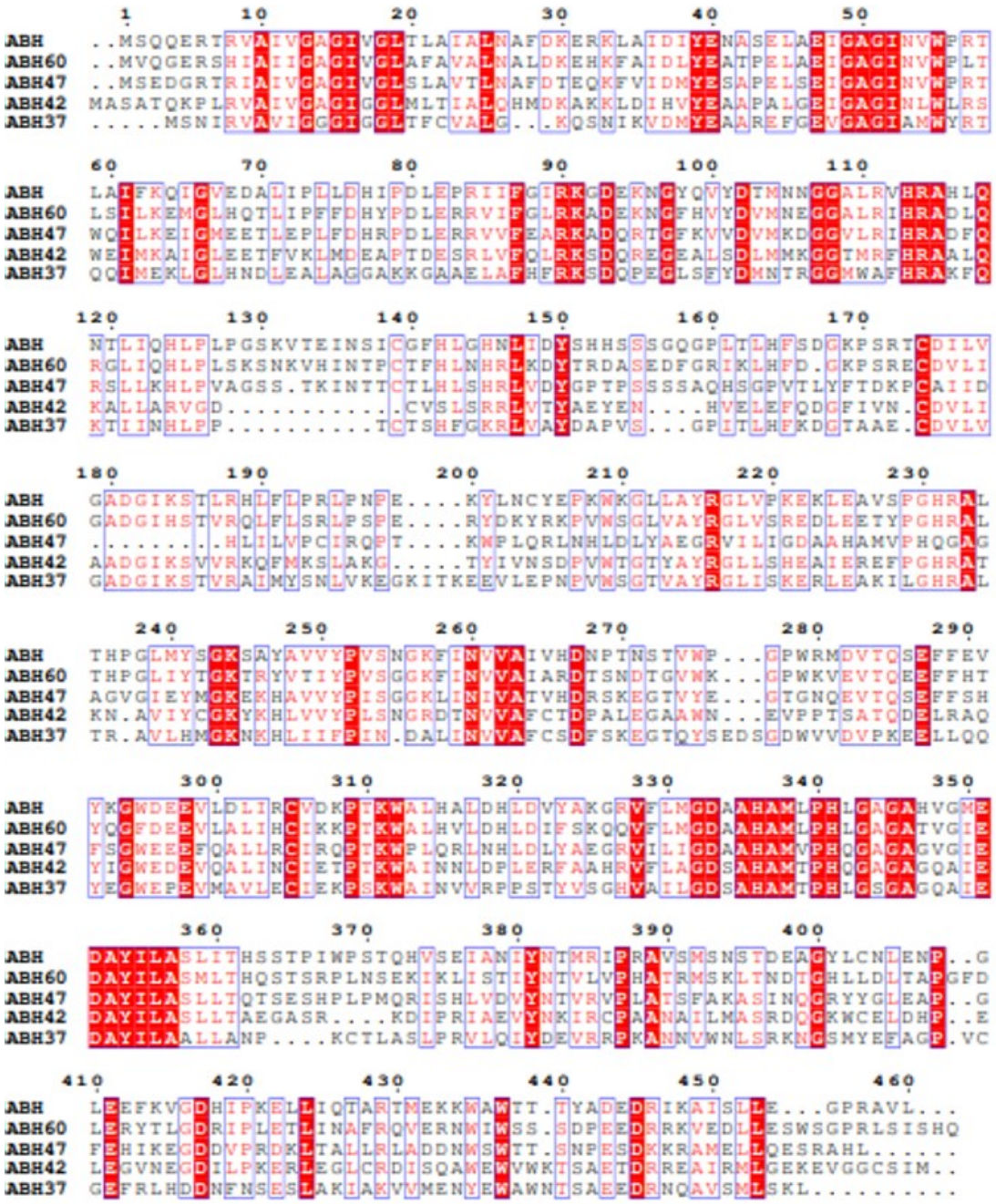


Figure S4. Sequence alignment of ABH (BAA07468.1) with ABH37 from *Fibularhizoctonia* sp. CBS 109695 (RXW22381.1), ABH42 from *Psathyrella aberdarensis* (RXW22381.1), ABH47 from *Leucoagaricus sp. SymC.cos* (KXN83799.1), and ABH60 from *Agaricus bisporus var. bisporus* H97 (XP_006456258.1).

Table S5. Schematic of strains harboring constructed plasmids for APAP biosynthesis. Gray filled rectangle: AroF gene; brown filled rectangle: PabAB gene; green filled rectangle: PabC gene; purple filled rectangle: PANAT gene; red filled rectangle: ABH or ABH homologous protein or ABH mutants; light purple rectangle: ribosome binding site (RBS). Recombinant *E. coli* PAB1, PAB2, PAB3, PAB5, PAB6, PAB7, PAB8, PAB9, PAB11 harboring constructed plasmids P1-P3-PB1, P1-P3-PB2, P1-P3-PB3, P1-P3-PB5, P1-P3-PB6, P1-P3-PB7, P1-P3-PB8, P1-P3-PB9, P1-P3-PB11, respectively.

Entry	Recombiant *E. coli* strains	plasmids
1	PAB1 (P1-P3-PB1)	P1 P15A AroF PabAB PabC P3 pBR322 PANAT PB1 ColE1 ABH37
2	PAB2 (P1-P3-PB2)	PB2 ColE1 ABH42
3	PAB3 (P1-P3-PB3)	PB3 ColE1 ABH47
4	PAB5 (P1-P3-PB5)	PB5 ColE ABH60^{T226V}
5	PAB6 (P1-P3-PB6)	PB6 ColE ABH60^{G272V}
6	PAB7 (P1-P3-PB7)	PB7 ColE ABH60^{G290V}
7	PAB8 (P1-P3-PB8)	PB8 ColE1 ABH60$^{T226V/T272V}$
8	PAB9 (P1-P3-PB9)	PB9 ColE1 ABH60$^{T226V/T290V}$
9	PAB11 (P1-P3-PB11)	PB11 ColE1 ABH60$^{T226V/G272V/T290V}$

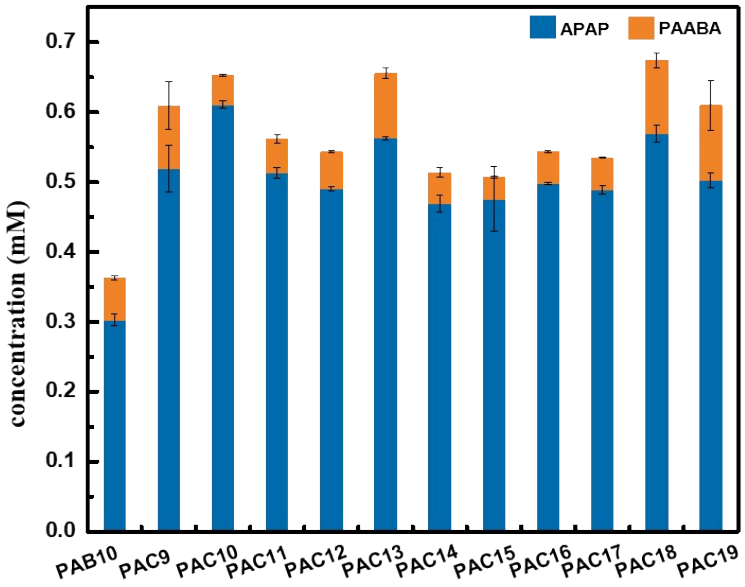


Figure S5. Titers of APAP: PAABA in PANAT strain and PANAT K211 (V, L, I, S, T, C, M, N, Q, and R respectively) mutant strains. Blue column: APAP, orange column: PAABA. PAB10 (PANAT); PAC9 (PANAT^{K211V}); PAC10 (PANAT^{K211L}); PAC11 (PANAT^{K211I}); PAC12 (PANAT^{K211P}); PAC13 (PANAT^{K211S}); PAC14 (PANAT^{K211T}); PAC15 (PANAT^{K211C}); PAC16 (PANAT^{K211M}); PAC17 (PANAT^{K211N}); PAC18 (PANAT^{K211Q}); PAC19 (PANAT^{K211R}). The data shown in Figure S5 are from three experiment replicates and are expressed as the mean value ± SD.

Table S6. Titers of APAP in strains PAC5-PAC19.

Entry	Strains	APAP: PAABA	APAP production (mM, mean value)	APAP production (mg/L, mean value)
1	PAC5 (K231W)	21.7	0.4699	71.03
2	PAC6 (K231F)	20	0.5077	76.74
3	PAC7 (K231H)	19.81	0.4884	73.83
4	PAC8 (K231Y)	19.85	0.4756	71.89
5	PAC9 (K231V)	5.76	0.5189	78.44
6	PAC10 (K231L)	14.38	0.6105	92.28
7	PAC11 (K231I)	10.58	0.5131	77.56
8	PAC12 (K231P)	9.25	0.49	74.07
9	PAC13 (K231S)	6.07	0.5627	85.06
10	PAC14 (K231T)	10.52	0.469	70.89
11	PAC15 (K231C)	15.1	0.4757	71.91
12	PAC16 (K231M)	11.06	0.4981	75.29
13	PAC17 (K231N)	10.62	0.4889	73.90
14	PAC18 (K231Q)	5.41	0.5692	86.04
15	PAC19 (K231R)	4.68	0.5025	75.96

Table S7. Kinetic parameters of PANAT and PANAT^{K211G}. The data shown in Table S7 are from three experiment replicates and are expressed as the mean value ±SD.

Substrates	Enzymes	K_m (μM^{-1})	K_{cat} (S^{-1})	K_{cat}/Km ($\mu M^{-1} S^{-1}$)
PABA	PANAT	613.51±6.8	0.20	$3.21x10^{-4}$
	PANAT^{K211G}	1014.67±11.34	0.05	$4.63x10^{-5}$
4-AP	PANAT	596.69±3.66	0.59	$9.84x10^{-4}$
	PANAT^{K211G}	473.80±5.93	1.41	$2.97 x10^{-3}$

Table S8. Schematic of strains (PAB10, PAD5 and PAD4) harboring constructed plasmids for APAP biosynthesis. Gray filled rectangle: AroF gene; brown filled rectangle: PabAB gene; green filled rectangle: PabC gene; purple filled rectangle: PANAT gene; red filled rectangle: $ABH^{G272V/T290V}$; dark green rectangle: EmrE; navy blue rectangle: AaeABRX; light purple rectangle: ribosome binding site (RBS). Recombinant *E. coli* PAB10, PAD5, PAD4 harboring constructed plasmids P1-P3-PB10, P1-P3-PDE, P1-P3-PD4, respectively.

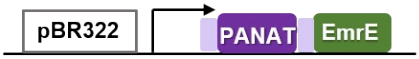
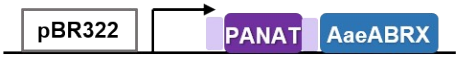
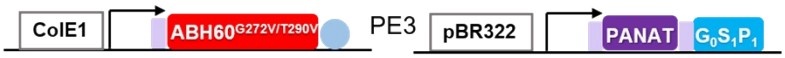
Entry	Recombiant *E. coli* strains	plasmids	
1	PAB10 (P1-P3-PB10)	P1	
		PB10	
2	PAD5 (P1-P3-PDE)	PDE	
3	PAD4 (P1-P3-PD4)	PD4	

Table S9. Schematic of strains harboring constructed plasmids for APAP biosynthesis. Gray filled rectangle: AroF gene; brown filled rectangle: PabAB gene; green filled rectangle: PabC gene; purple filled rectangle: PANAT gene; red filled rectangle: $ABH^{G272V/T290V}$; blue rectangle: scaffolds $GBD_xSH3_yPDZ_z$; light purple rectangle: ribosome binding site (RBS). Yellow triangle is the ligand to PDZ domain. Light blue round is the ligand to SH3 domain. Recombinant *E. coli* PAB10, PAE1, PAE2, PAE3, PAE4, PAE5 harboring constructed plasmids P1-P3-PB10, PE1-PE2-PE3, PE1-PE2-PE4, PE1-PE2-PE5, PE1-PE2-PE6, PE1-PE2-PE7, respectively.

Entry	Recombiant *E. coli* strain	plasmids	
1	PAB10 (P1-P3-PB10)	P1	
		PB10	
2	PAE1 (PE1-PE2-PE3)	PE1	
		PE2	
3	PAE2 (PE1-PE2-PE4)	PE4	
4	PAE3 (PE1-PE2-PE5)	PE5	
5	PAE4 (PE1-PE2-PE6)	PE6	
6	PAE5 (PE1-PE2-PE7)	PE7	

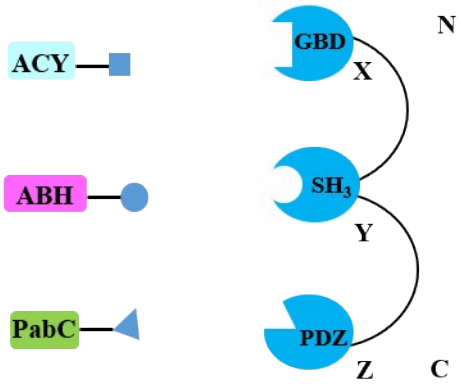


Figure S6. The diagram for the APAP biosynthetic key enzymes scaffolding strategy. The GBD domain (GTPase binding domain) is from the actin polymerization switch N-WASP, the SH3 domain (Src homology 3 domain) is from the adaptor protein CRK), and the PDZ (PSD95/DlgA/Zo-1) domain is from the adaptor protein syntrophin. These domains were linked by flexible nine-residue glycine-serine linkers to construct scaffolds $GBD_xSH3_yPDZ_z$. ACY: PANAT (Arylamine N-acetyltransferase), ABH: 4-aminobenzoate hydroxylase, PabC: 4-amino-4-deoxychorismic acid lyase. Rectangle is the ligand to GBD, Circle is the ligand to SH3 domain, triangle is the ligand to PDZ domain.

a

Entry	Recombiant *E. coli* strain		plasmids
1	PADF1 (PF3-PE2-PF4)	PF1	P15A — AroF — PabAB — PabC
		PE2	ColE1 — $ABH60^{G272V/T290V}$
		PF4	pBR322 — $PANAT_{K211G}$ — EmrE — AaeABRX — $G_1S_2P_2$
3	PADF3 (PF5-PF6-PF7)	PF5	pBR322 — AroF — PabAB — PabC
		PF6	pMB1 — $ABH60^{G272V/T290V}$ — $PANAT^{K211G}$
		PF7	ColE1 — EmrE — AaeABRX — $G_1S_2P_2$
4	PADF4 (PF7-PF8-PF9)	PF8	ColE1 — $ABH60^{G272V/T290V}$ — AroF — PabAB — PabC
		PF9	pBR322 — $PANAT^{K211G}$
		PF10	P15A — EmrE — AaeABRX — $G_1S_2P_2$
5	PADF5 (PF11-PF9-PF10)	PF11	ColE1 — AroF — PabAB — PabC — $ABH60^{G272V/T290V}$
6	PADF6 (PF1-PF12-PF13)	PF12	ColE1 — $ABH60^{G272V/T290V}$ — $PANAT^{K211G}$
		PF13	pBR322 — EmrE — AaeABRX — $G_1S_2P_2$

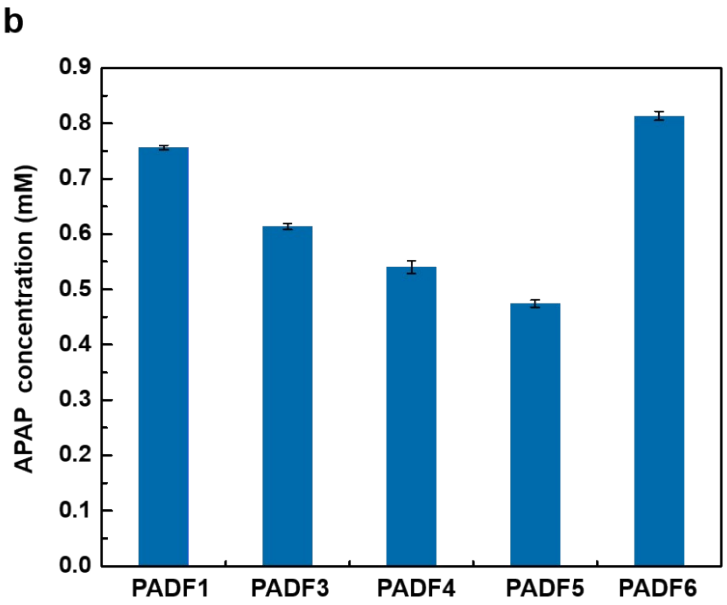


Figure S7. Improvement of APAP titers by gene module combinations. a) Schematic of strains harboring constructed plasmids for APAP biosynthesis. b) Engineered *E. coli* cells (PADF1, PADF3, PADF4, PADF5, and PADF6) for the production of APAP. P15A: pACYC-Duet-1; pBR322: pET-28a; ColE1: pET-Duet-1; arrow: T7 promoter. Gray filled rectangle: AroF gene; brown filled rectangle: PabAB gene; green filled rectangle: PabC gene; purple filled rectangle: $PANAT^{K231G}$ gene; red filled rectangle: $ABH60^{G272V/T290V}$ gene, dark green rectangle: EmrE; navy blue rectangle: AaeABRX; bule rectangle: $G_1S_2P_2$; light purple rectangle: ribosome binding site (RBS). Yellow triangle is the ligand to PDZ domain. Light blue round is the ligand to SH3 domain. The data shown in b are from three experiment replicates and are expressed as the mean value ± SD.

a

Entry	Recombiant *E. coli* strains	plasmids
1	PA2 (P4)	P4 ColE1 ABH PANAT AroF PabAB PabC
2	PA3 (P5)	P5 ColE1 ABH AroF PabAB PabC PANAT

b

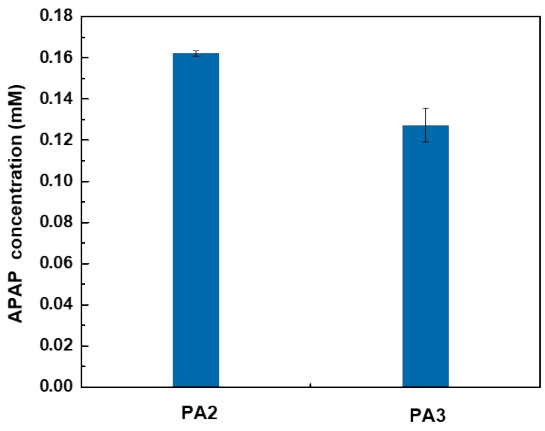
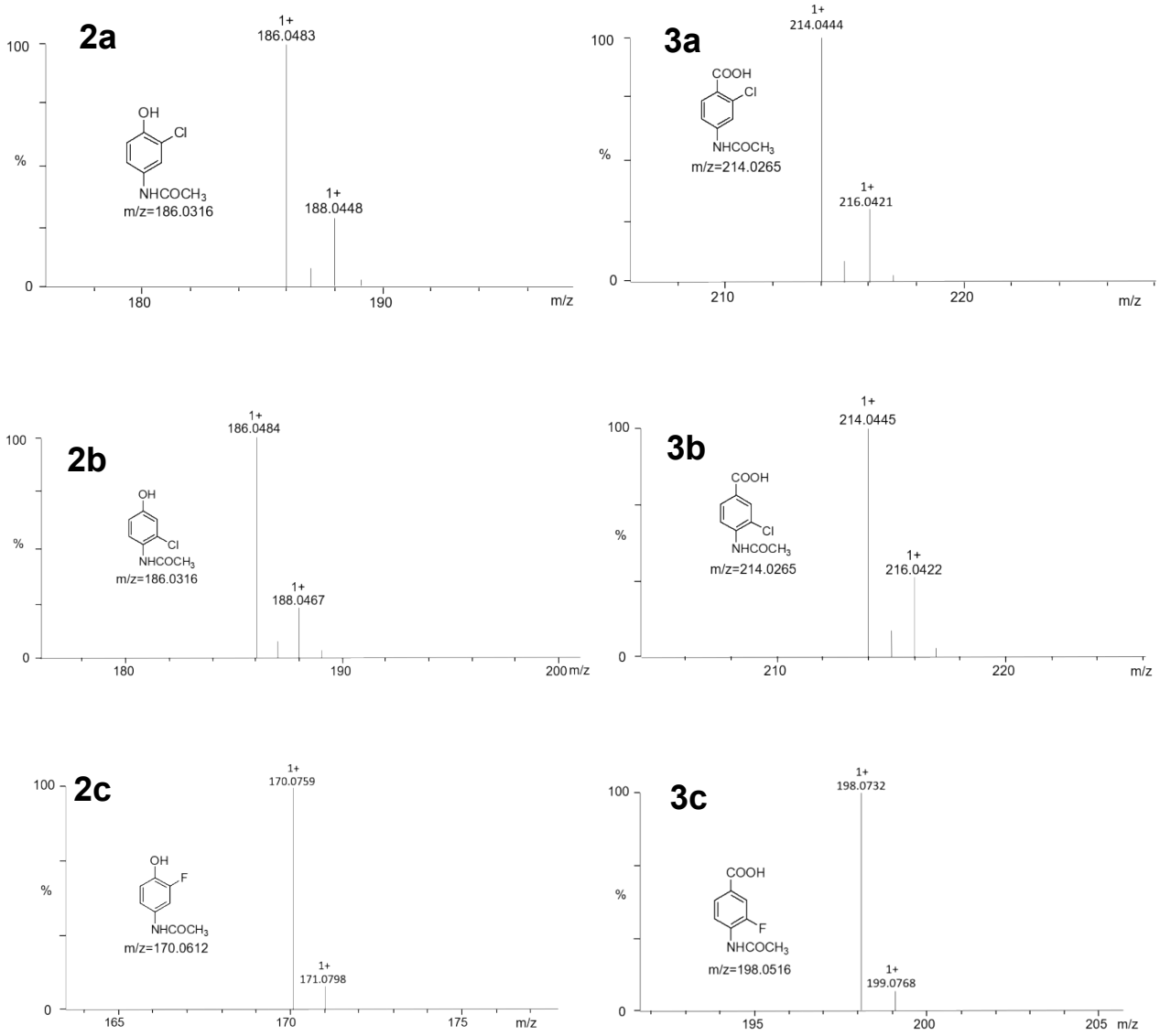


Figure S8. *In vivo* production of APAP (1) in *E. coli* PA2 and PA3 using the gene clusters P4 and P5 respectively, measured by HPLC after 48h. a) Construction of *E. coli* cells expressing gene clusters (containing AroF, PabAB, PabC, PANAT, ABH). b) Engineered *E. coli* PA2 and PA3 for the production of APAP (**1**). ColE1: pET-Duet-1; arrow: T7 promoter. Proteins: AroF, DAHP synthetase feedback inhibited by tyrosine; PabAB, *p*-aminobenzoic acid synthase; PabC, 4ADC lyase; ABH, 4-aminobenzoate hydroxylase; PANAT, arylamine N-acetyltransferase. Gray filled rectangle: AroF gene; brown filled rectangle: PabAB gene; green filled rectangle: PabC gene; purple filled rectangle: PANAT gene; red filled rectangle: ABH; light purple rectangle: ribosome binding site (RBS). Recombinant *E. coli* PA2, PA3 harboring constructed plasmids P4, P5, respectively. The data shown in b are from three experiment replicates and are expressed as the mean value ± SD.



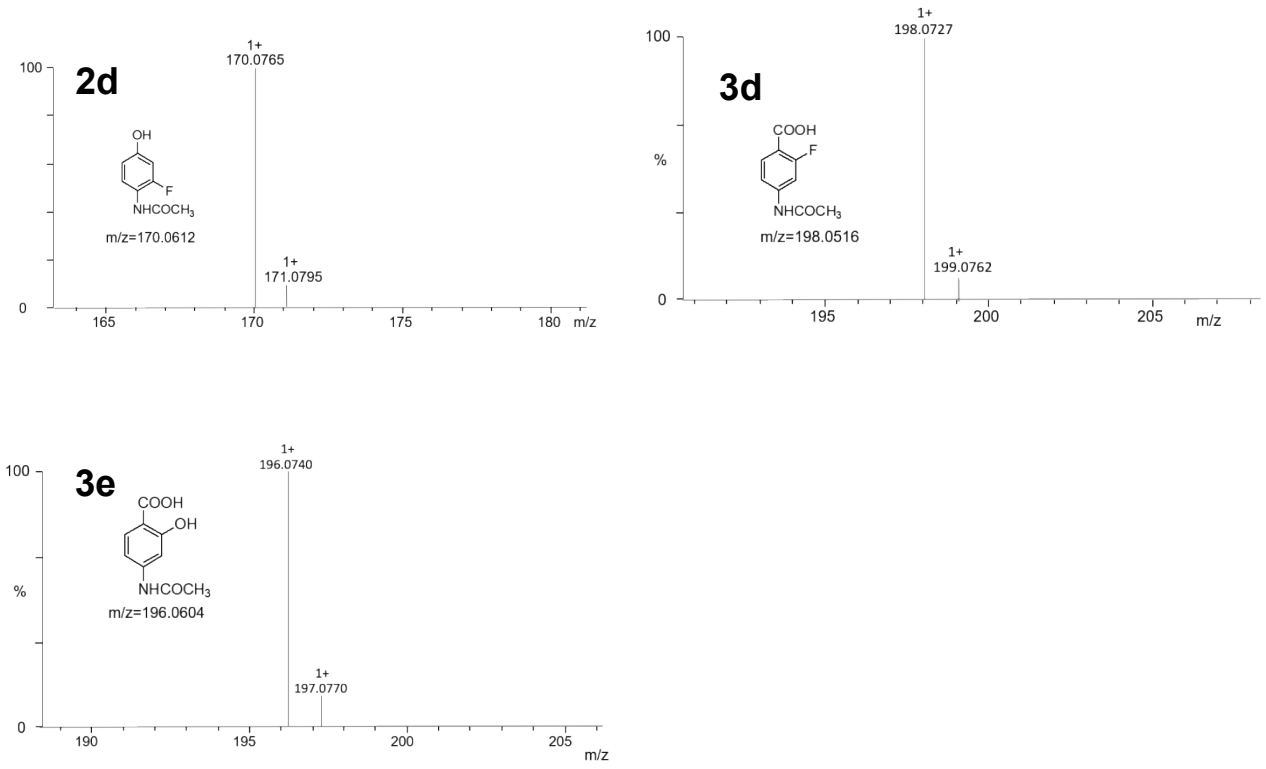


Figure S9. Characterization of **2a**, **3a**, **2b**, **3b**, **2c**, **3c**, **2d**, **3d**, **3e** by ESI-MS.

Table S10. The amino acid sequence of GBD domain, the SH3 domain, and the PDZ domain.

GBD
TKADIGTPSNFQHIGHVGWDPNTGFDLNNLDPELKNLFDMCGISEAQLKDRETSKVIYDFIEKTG GVEAVKNELRRQAP
SH3
AEYVRALFDFNGNDEEDLPFKKGDILRIRDKPEEQWWNAEDSEGKRGMIPVPYVEKY
PDZ
LQRRRVTVRKADAGGLGISIKGGRENKMPILISKIFKGLAADQTEALFVGDAILSVNGEDLSSA THDEAVQALKKTGKEVVLEVKYMKEVSPYFK

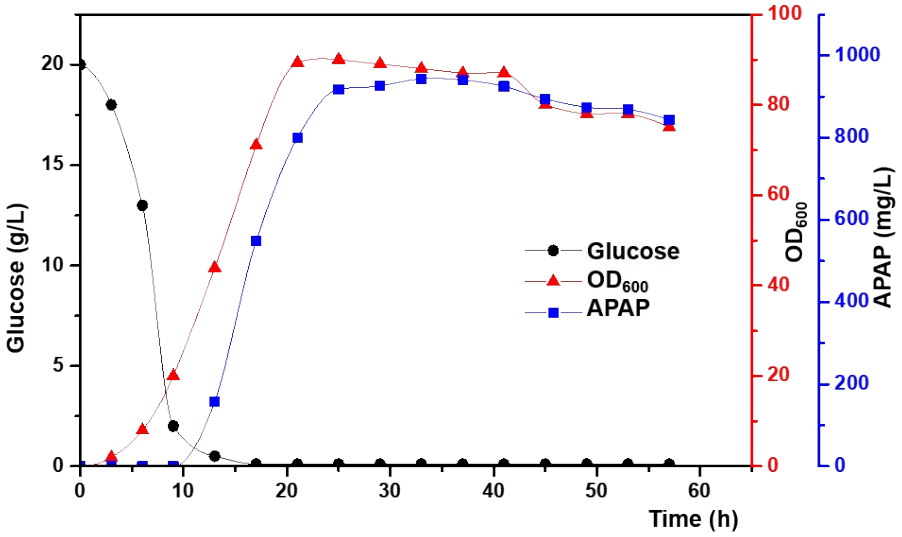


Figure S10. Fed-batch fermentation of PADF2. The strain PADF2 was inoculated into the fermentation medium with 20 g/L glucose (as described in section 2.9). When the cell density reached to an OD_{600} of 20 after nine hours of cultivation (The residual sugar concentration was about 2 g/L), the recombinant proteins were induced by 0.1 mM IPTG.

Dissolved oxygen level was maintained at 30% through automatic control of the agitation speed varying from 400 to 1000 rpm. The pH was regulated by the addition of $NH_3 \cdot H_2O$. Glucose (400 g/L) was added to maintain a low residual concentration of about 200 mg/L to avoid glucose inhibition. OD_{600} was marked with red triangle, residual glucose was marked with black circle, and APAP was marked with blue square.

References

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