A Synthetic Recursive "+1" Pathway for Carbon Chain Elongation

Supplemental Information

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Supplemental Methods.

Primer Sequences

LeuAsalfwd: CGGTCGACAGAGGAGAAAGGTACCATGAGCCAGCAAGTCATTATTTTCG
LeuAxbarev: CGTCTAGATTACACGGTTTCCTTGTTGTTTTC

SalI and XbaI restriction sites are underlined.

Supplemental Figures and Tables.

Table S1. Energy scores, ligand binding scores, and packing scores of LeuA-substrate models calculated by RosettaDesign

LeuA mutant	Substrate	Energy Score	Ligand Binding Score	Packing Score
G462D	Ketoisovalerate	-554.8	2.4	0.494
	2-ketobutyrate	-555.3	3.3	0.494
	2-ketovalerate	-533.9	4.1	0.502
	2-ketocaproate	-517.9	18.2	0.495
	2-ketoheptanoate	-344.4	86.9	0.504
	2-ketooctanoate	252.4	276.7	0.495
	Phenylpyruvate	-455.2	46.7	0.496
	Homophenylpyruvate	-427.0	58.6	0.494
H97A, S139G, N167G, P169A, G462D	Ketoisovalerate	-543.2	2.6	0.488
	2-ketobutyrate	-544.0	3.4	0.489
	2-ketovalerate	-528.2	3.0	0.495
	2-ketocaproate	-544.9	2.9	0.485
	2-ketoheptanoate	-528.7	2.1	0.495
	2-ketooctanoate	-372.6	27.6	0.487
	Phenylpyruvate	-545.6	2.6	0.487
	Homophenylpyruvate	-721.0	1.3	0.494

Figure S1.

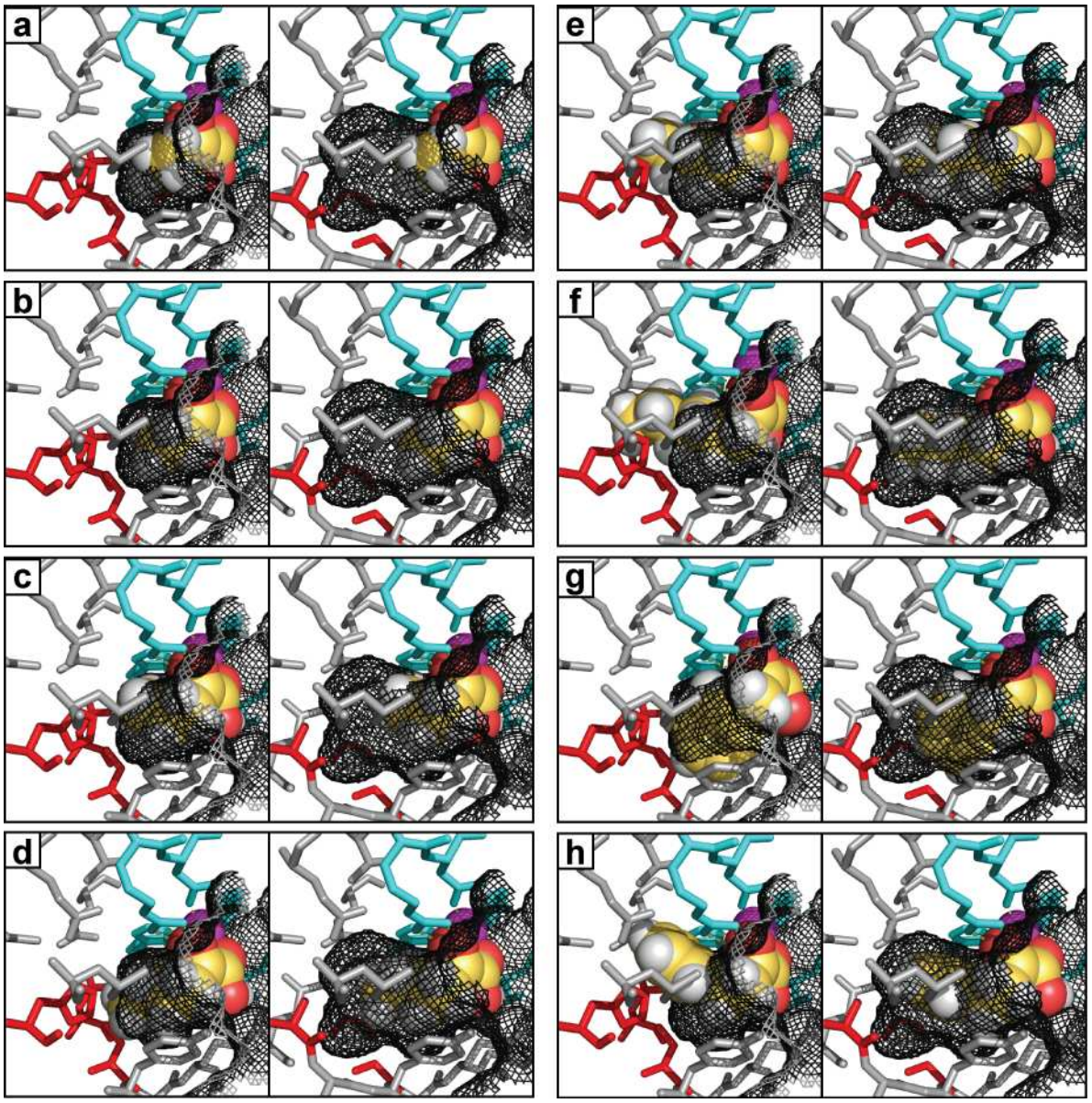

Figure S1. Protein-substrate molecular complex models. **(a-h)** *G462D* enzyme active site models are shown on the left and quintuple mutant enzyme active site models are shown on the right. Protein residues, metal ion, and coordination interactions are shown and colored as in Figure 2. Substrate binding pockets are shown as wire mesh. Substrates present in the models are: **(a)** KIV **(b)** 2-ketobutyrate **(c)** 2-ketovalerate **(d)** 2-ketocaproate **(e)** 2-ketoheptanoate **(f)** 2-ketooctanoate, **(g)** phenylpyruvate **(h)** homophenylpyruvate.

Figure S2.

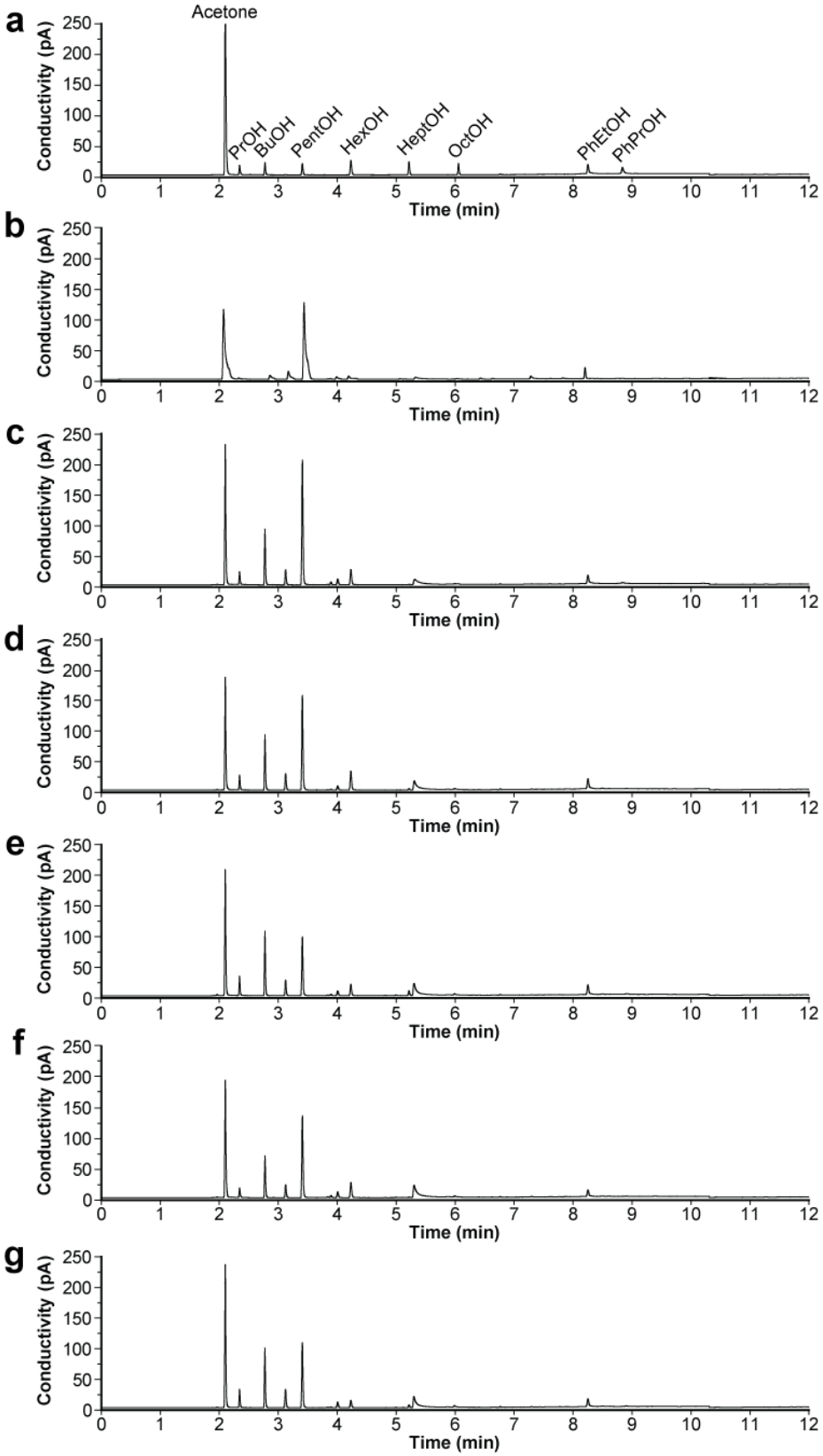
a
b
c
d
e
f
g
Acetone
PrOH
BuOH
PentOH
HexOH
HeptOH
OctOH
PhEtOH
PhPrOH
Conductivity (pA)
Time (min)

Figure S2. Representative GC-FID data for *E. coli* fermentations using various *Ec*LeuA mutants. Acetone was used as an internal standard in all cases. Quantification of alcohols produced is presented in Table 4. **(a)** Alcohol standards in water consisting of approximately 160 mg l^{-1} 1-propanol (PrOH), 162 mg l^{-1} 1-butanol (BuOH), 163 mg l^{-1} 1-pentanol (PentOH), 163 mg l^{-1} 1-hexanol (HexOH), 164 mg l^{-1} 1-heptanol (HeptOH), 165 mg l^{-1} 1-octanol (OctOH), 204 mg l^{-1} 2-phenylethanol (PhEtOH), and 202 mg l^{-1} 3-phenylpropanol (PhPrOH). **(b-g)** Alcohol products obtained when using: **(b)** *G462D Ec*LeuA **(c)** *S139G/G462D Ec*LeuA **(d)** *S139G/N167A/G462D Ec*LeuA **(e)** *H97A/S139G/G462D Ec*LeuA **(f)** *H97A/S139G/N167A/G462D Ec*LeuA **(g)** *H97A/S139G/N167G/G462D Ec*LeuA.

Figure S3.

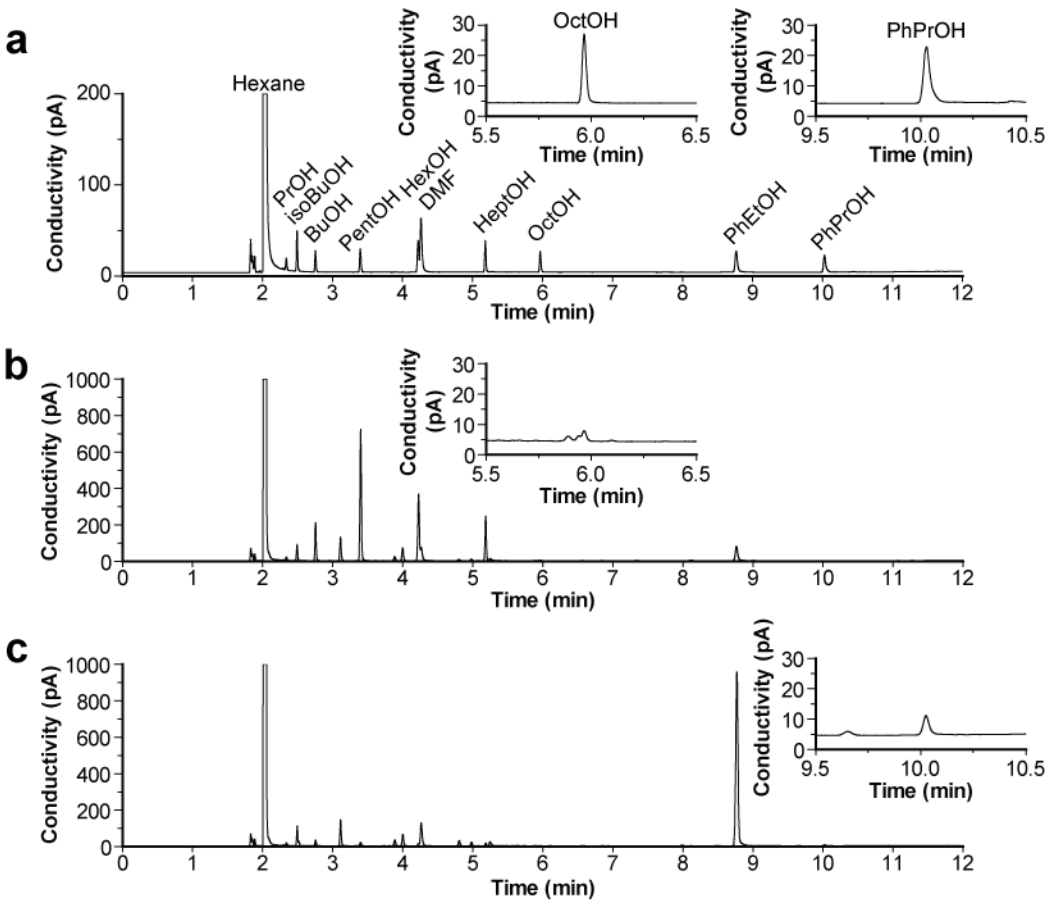

Figure S3. Representative GC-FID data for *E. coli* fermentations using the *H97A/S139G/N167G/P169A/G462D Ec*LeuA mutant. Alcohols produced were extracted from fermentation mixtures with n-hexane. 2,5-dimethylfuran (DMF) was used as an internal standard in all cases. Quantification of alcohols produced is presented in Table 4. **(a)** Alcohol standards in n-hexane consisting of approximately 160 mg l^{-1} 1-propanol (PrOH), 160 mg l^{-1} isobutanol (isoBuOH), 162 mg l^{-1} 1-butanol (BuOH), 163 mg l^{-1} 1-pentanol (PentOH), 163 mg l^{-1} 1-hexanol (HexOH), 164 mg l^{-1} 1-heptanol (HeptOH), 165 mg l^{-1} 1-octanol (OctOH), 204 mg l^{-1} 2-phenylethanol (PhEtOH), and 202 mg l^{-1} 3-phenylpropanol (PhPrOH). Insets show magnified regions of the GC-FID trace around OctOH and PhPrOH. **(b)** Alcohol products obtained in threonine overproduction strain ATCC98082 *ΔrhtA*. Inset shows magnified region around OctOH. **(c)** Alcohol products obtained in phenylalanine overproduction strain ATCC31884. Inset shows magnified region around PhPrOH.