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Supplementary Materials for

Exploring alternative pathways for the in vitro establishment of the HOPAC cycle for synthetic CO_2 fixation

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Legend for data S1
Amino Acid Sequences
References

Other Supplementary Material for this manuscript includes the following:

Data S1

Supplemental Table 1. Concentration of enzymes and cofactors in reductive pathway assays. Cells highlighted in grey are in mM, all others are in μM.

	L-Glutamate	L-Alanine	Pcs	Pcl
MOPS pH 7.5	50	50	50	50
$NaHCO_3$	50	50	50	50
Formate	20	20	20	20
NH_4Cl	100	100	-	-
ATP	-	-	2	2
NADPH	2	2	2	2
L-Glutamate	250	-	-	-
L-Alanine	-	250	-	-
$MgCl_2$	-	-	5	5
CoA	-	-	1	1
$PolyPO_4$	-	-	25	25
Gdh	25	-	-	-
Adh	-	25	-	-
Fdh	30	30	30	30
Ppk_{Sm}	-	-	50	50
Ppk_{Aj}	-	-	50	-
Mcr_{CT}	20	20	-	-
GabT	60	-	-	-
βapt	-	60	-	-
βact	50	50	-	-
βCal	50	50	-	-
Ccr	25	25	-	-
Pcs	-	-	150	-
Hps	-	-	-	50
Ech	-	-	-	50
Ccr	-	-	-	50
Mcr	-	-	50	50
3act	-	-	-	-
Pcc	-	-	150	-
Malonyl-CoA	1	1	1	1
Acetyl-CoA	2	2	-	-

Supplemental Table 2. Concentration of enzymes and cofactors in oxidative pathway assays. Cells highlighted in grey are in mM, all others are in µM.

	Mcd	Scs	Sct	Sch
MOPS pH 7.5	50	50	50	50
ATP	-	2.5	-	2.5
ADP	-	2.5	-	2.5
$MgCl_2$	5	5	5	5
CoA	0	0.5	0	0.5
AdoCbl	65	65	65	65
FAD	30	30	30	30
Ascorbic Acid	3.375	3.375	3.375	3.375
Ferrocenium	-	1.5	1.5	1.5
Mcm	5	5	5	5
Epi	5	5	5	5
Mch	10	10	10	10
Mcl	40	40	40	40
Mcd	20	-	-	-
EtfAB	20	-	-	-
QO	60	-	-	-
SdhA/B	-	100	100	100
FumC	-	10	10	10
SucCD	-	100	-	-
Mcs	-	100	-	100
Smt	-	-	20	-
Malate	-	-	0.2	-
Sch	-	-	-	1
Methylmalonyl-CoA	1	1	1	1

Supplemental Table 3. Concentration of enzymes and cofactors in full HOPAC cycle assays. Cells highlighted in grey are in mM, all others are in μM.

	$HOPAC_{Ccr}$ 1.0	$HOPAC_{Ccr}$ 2.0	$HOPAC_{Ccr}$ 3.0	$HOPAC_{Ccr}$ 3.1	$HOPAC_{Pcs/Pcc}$
MOPS pH 7.5	50	50	50	50	50
$NaHCO_3$ ^{13}C	33	33	33	33	33
Formate ^{13}C	13	13	13	13	13
ATP	3	3	3	3	3
NADPH	3	3	3	3	3
NADH	2	2	2	2	2
$MgCl_2$	5	5	5	5	5
CoA	0.5	0.5	0.5	0.5	0.5
$PolyPO_4$	13	13	13	13	13
AdoCbl	65	65	65	65	65
FAD	30	30	30	30	30
Ascorbic Acid	3.375	3.375	3.375	3.375	3.375
CoADSR	15	15	15	15	15
Carbonic Anhydrase	5	5	5	5	5
SodB	5	5	5	5	5
KatE	35	35	35	35	35
Fdh	50	50	50	50	50
Ppk_{Sm}	50	50	50	50	50
Ppk_{Aj}	-	-	-	-	20
Pcs	-	-	-	-	50
Pcc	-	-	-	-	20
Pcl	40	40	40	40	-
Ech	20	20	20	20	-
Ccr	50	50	50	50	-
Mcm	5	5	5	5[a]	5[a]
Epi	5	5	5	5	5
Mcd	20	20	20	20	20
Etf	20	20	20	20	20
EtfQO	-	60	60	60	60
Mch	10	10	10	10	10
Mcl	50	50	50	50	50
Grd	30	30	30	30	30
$Pcc_{(D407I)}$	20	20	20	20	20
Mcr	5	5	5	5	5
MeaB	-	-	50[b]	-	-
Acetyl-CoA	200	200	200	200	200

a. For Mcm supplementation, the assay was initiated with 1.25 μM Mcm rather than the 5 μM of other assays. Additional aliquots of 1.25 μM (adjusted to the new volume after time points were removed) until a net concentration of 5 μM had been added. b. For assays including the chaperone MeaB, Mcm was pre-incubated with MeaB to allow interaction to occur prior to exposure to methylmalonyl-CoA.

Supplemental Table 4. Concentrations for METIS optimization. Values are μM except for shaded cells which are mM.

MOPS	25	68.75	113	156.3	200
ATP	1	3.25	5.5	7.75	10
GTP	1	4	7	10	
NADPH	1	3.25	5.5	7.75	10
NADH	1	4	7	10	
$MgCl_2$	0	25	50	75	100
CoA	0	0.5	1	1.5	2
$NaHCO_3$	25	67.5	113	157.5	200
Formate	10	32.5	55	77.5	100
CP	2.5	10	30	60	
B12	0.1	0.325	0.55	0.8	1
FAD	0.1	0.325	0.55	0.8	1
Ascorbate	0	2.5	5	7.5	10
Dsr	0.3	2.7	5	7.4	9.75
Carbonic Anhydrase	0.25	0.5	0.75	1	
SOD	0.25	0.5	0.75	1	
KatE	1.4	3.4	5.4	7.5	9.5
Fdh	3.3	8.4	13.4	18.4	23.4
Creatine Kinase	0.4	1.2	2.4	3.4	
Pcl	1.5	6.1	12.2	18.3	24.4
Ech	1.4	2.3	3.2	4.1	4.9
Ccr	1.6	2.4	3.1	3.9	4.7
Mcm	2.1	4.2	6.3	8.5	
MeaB	1.9	23.3	48.5	73.8	99.1
Epi	0.78	2.3	4.6	7	9.3
Mcd	2.3	25.4	48.5	99.3	
Etf	3.9	27	50	73.2	96.3
QO	5.8	29.1	52.3	75.6	98.9
Mch	0.47	2.8	5.2	7.5	9.8
Mcl	2.8	5.6	8.4		
$Pcc_{(D407I)}$	0.8	3.3	5.7	8.2	9.8
Mcr	1.2	3.7	6.1	9.7	
Grd	5.6	9	12.4	15.8	19.2
AhpFC	0.9	2.9	5.2	7.5	9.9
CoAE	0.75	3	5.25	7.5	9.75
YjeF	0.9	3.4	5.1	6.8	9.4

Supplemental Table 5. Parameters used for the detection of glycolate by LCMS.

Compound	Quantifier	Collision Energy	Qualifier	Collision Energy	Dwell	Fragmenter voltage	Cell Accelerater Volatege
X1	75→75	0	75→47	9	150	380	5
X2	77→77	0	77→48	9	150	380	5

Supplemental Table 6. Enzyme List

Abr.	Enzyme Name	Host Organism	Primary reaction in this study	K_m	V_{max} (U mg^{-1})	Additional Data
Ccr	crotonyl-CoA carboxylase/reductase	*Methylorubrum extorquens*	acrylyl-CoA + CO_2 + NADPH = (2S)-methylmalonyl-CoA + $NADP^+$	780 µM acrylyl-CoA 210 µM NADPH	274.4	
Epi	methylmalonyl-CoA epimerase	*Cereibacter sphaeroides*	(2S)-methylmalonyl-CoA = (2R)-methylmalonyl-CoA	80 µM methylmalonyl-CoA	440[27]	
Mcm	methylmalonyl-CoA mutase	*Cereibacter sphaeroides*	(2R)-methylmalonyl-CoA = succinyl-CoA	19 µM methylmalonyl-CoA	450	
PmMcd	methylsuccinyl-CoA dehydrogenase	*Pseudomonas migulae*	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	78 µM succinyl-CoA	5.2	49 Umg^{-1} 81 µM methylsuccinyl-CoA
CsMcd	methylsuccinyl-CoA dehydrogenase	*Cereibacter sphaeroides*	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	94 µM succinyl-CoA	0.82	71 Umg^{-1} 41 µM methylsuccinyl-CoA
SaMcd	methylsuccinyl-CoA dehydrogenase	*Streptomyces albus*	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	39 µM succinyl-CoA	0.29	17 Umg^{-1} 12 µM methylsuccinyl-CoA
WmMcd	methylsuccinyl-CoA dehydrogenase	*Wenxinia marina*	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	30 µM succinyl-CoA	0.35	86 Umg^{-1} 29 µM methylsuccinyl-CoA
CvMcd	methylsuccinyl-CoA dehydrogenase	*Caulobacter vibrioides*	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	61 µM succinyl-CoA	1.0	131 Umg^{-1} 55 µM methylsuccinyl-CoA
ElMcd	methylsuccinyl-CoA dehydrogenase	*Erythrobacter litoralis*	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	32 µM succinyl-CoA	2.8	18 Umg^{-1} 67 µM methylsuccinyl-CoA
FsMcd	methylsuccinyl-CoA dehydrogenase	*Frankia sp.*	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	38 µM succinyl-CoA	1.7	39 Umg^{-1} 43 µM methylsuccinyl-CoA
PdMcd	methylsuccinyl-CoA dehydrogenase	*Paracoccus denitrificans*	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	101 µM succinyl-CoA	1.7	88 Umg^{-1} 88 µM methylsuccinyl-CoA
HrMcd	methylsuccinyl-CoA dehydrogenase	Bacterium HR19	succinyl-CoA + FAD = fumaryl-CoA + $FADH_2$	99 µM succinyl-CoA	1.4	1.1 Umg^{-1} 723 µM methylsuccinyl-CoA
Mch	mesaconyl-CoA hydratase	*Cereibacter sphaeroides*	fumaryl-CoA + H2O = malyl-CoA	280 µM fumaryl-CoA	$1.7x10^3$	18 Umg^{-1} 80 µM (S)-malyl-CoA 59 Umg^{-1} 210 µM (2R,3S)-β-methylmalyl-CoA
Mcl	malyl-CoA lyase	*Cereibacter sphaeroides*	malyl-CoA = acetyl-CoA + glyoxylate	40 µM (S)-malyl-CoA	6.3	9.7 Umg^{-1} 110 µM (2R,3S)-β-methylmalyl-CoA
$Pcc_{(D407I)}$	propionyl-CoA carboxylase	*Methylorubrum extorquens*	acetyl-CoA + HCO_3^- + ATP = malonyl-CoA + ADP + P_i	560 µM acetyl-CoA 50 µM ATP	15.5	28 Umg^{-1} 140 µM propionyl-CoA
Mcr	malonyl-CoA reductase	*Chloroflexus aurantiacus*	malonyl-CoA + NADPH = malonic semialdehyde + $NADP^+$ + CoA malonic semialdehyde + NADPH = 3-hydroxypropionate + $NADP^+$	1.3 µM malonyl-CoA 10 µM NADPH	24	
Mcr-CT	Malonyl-CoA reductase c-terminal domain	*Chloroflexus aurantiacus*	malonyl-CoA + NADPH = malonic semialdehyde + $NADP^+$ + CoA	8 µM malonyl-CoA 39 µM NADPH	49	
Pcl	propionyl-CoA ligase	*Cupriavidus necator*	3-hydroxypropionate + ATP + CoA = 3-hydroxypropionyl-CoA + ADP + P_i	9.9 mM 3-hydroxypropionate 96 µM CoA 19 µM ATP	2.6	

Ech	enoyl-CoA hydratase	*Pseudomonas aeruginosa*	3-hydroxypropionyl-CoA = acrylyl-CoA + H_2O	330 μM acrylyl-CoA	720	
Pcs	propionyl-CoA synthetase	*Erythrobacter NAP1*	3-hydroxypropionate +CoA + ATP + NADPH + HCO_3^- = (2S)-methylmalonyl-CoA + AMP + PP_i + $NADP^+$ + H_2O	3-hydroxypropionate 85 μM 10 μM ATP 340 μM CoA 140 μM NADPH	0.14	
Pcc	propionyl-CoA carboxylase	*Methylorubrum extorquens*	propionyl-CoA + HCO_3^- + ATP = (2S)-malonyl-CoA + ADP + P_i	55 μM propionyl-CoA 330 μM ATP	22	1.1 Umg^{-1} 270 μM acetyl-CoA
Smt	succinyl-CoA:malate CoA transferase	*Chloroflexus aurantiacus*	succinyl-CoA + malate = succinate + malyl-CoA	0.96 mM succinyl-CoA 0.91 mM malate	78	
Sdh	succinate dehydrogenase	*Escherichia coli*	Succinate + FAD = fumarate + $FADH_2$	63 μM succinate	0.59	0.23 Umg^{-1} 86 μM succinate
βCal	β-alanyl-CoA:ammonia lyase	*Stigmatella aurantiaca*	β-alanyl-CoA = acrylyl-CoA + NH_3	70 μM β-alanyl-CoA	12	
Gdh	glutamate dehydrogenase	*Paracoccus denitrificans*	2-oxoglutarate + NH_3 + NADPH = L-glutamate + H_2O + $NADP^+$	390 μM 2-oxoglutarate 10.7 μM NADPH 86 mM NH_4	48	0.47 Umg^{-1} 490 μM glutamate 7 μM $NADP^+$
Scs	succinyl-CoA synthetase	*Escherichia coli*	succinyl-CoA + ADP + P_i = succinate + CoA + ATP	250 μM succinate 4.0 μM CoA 70 μM ATP	37.7[29]	
Sch	succinyl-CoA hydrolase	*Mus musculus*	succinyl-CoA + H_2O = succinate + CoA	1.8 mM succinyl-CoA	468	
GabT	4-aminobutyrate-2-oxoglutarate transaminase	*Escherichia coli*	malonic semialdehyde + L-glutamate = 2-oxoglutarate + β-alanine	98 μM malonic semialdehyde 7 μM L-glutamate	1.1	

Supplementary Table 7. X-ray diffraction data collection and model refinement statistics

Crystal	McdPm with bound FAD and (2*S*)-methylsuccinyl-CoA
PDB ID	8CIW
Data collection	
Beamline	DESY PETRA III P13
Wavelength (Å)	0.9763
Space Group	$C\ 2\ 2\ 2_1$
Unit cell dimensions	
a, b, c (Å)	139.80, 169.56, 118.39
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	25.01 – 1.93 (2.03 – 1.93)
Unique reflections	105292 (15053)
Multiplicity	13.2 (12.7)
Completeness (%)	99.7 (98.6)
$I / \sigma I$	14.6 (2.3)
R_{merge}	0.119 (1.165)
R_{pim}	0.034 (0.337)
$CC_{1/2}$	0.999 (0.892)
Refinement	
R_{work} / R_{free}	0.1739 / 0.2027
RMS bonds	0.0086
RMS angles	0.967
Ramachandran	
favored (%)	98.28
outliers (%)	0.00
Rotamer outliers (%)	0.47
Number of atoms	9225
Protein	8370
Ligands	288
Solvent	567
Average B-factor	39.78
Protein	39.51
Ligands	40.90
Solvent	43.27

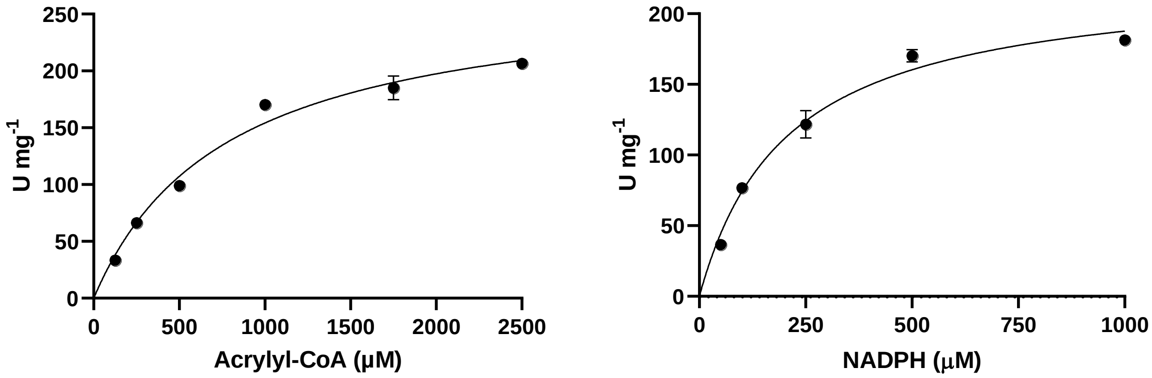
Values in parentheses are for highest-resolution shell.

Supplementary Table 8. CO_2 fixation comparrison of the HOPAC cycle with other synthetic cycles and the Calvin cycle.

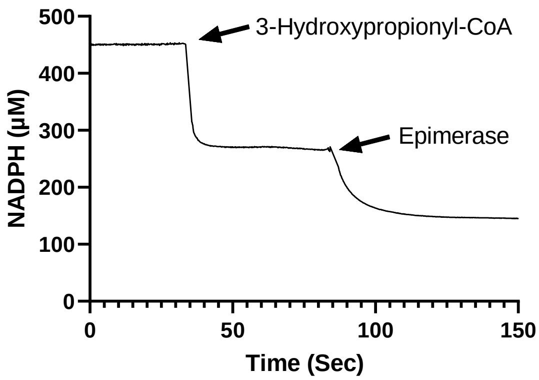
Cycle	**Rate (nmol CO_2 min^{-1} mg^{-1} CO_2-fixing enzymes)**	**ATP Requirements**	**NAD(P)H Requirements**	**Cycle Product**	**Reference**
HOPAC	2.4	2	3	Glyoxylate	This study
CETCH	5	1	4	Glyoxylate	[1]
THETA	2.7	4	5	Acetyl-CoA	
POAP	8.0	3	1	Oxalate	[50]
CBB	1-3	9	6	glyceraldehyde-3-phosphate	[51]

Supplementary Figures

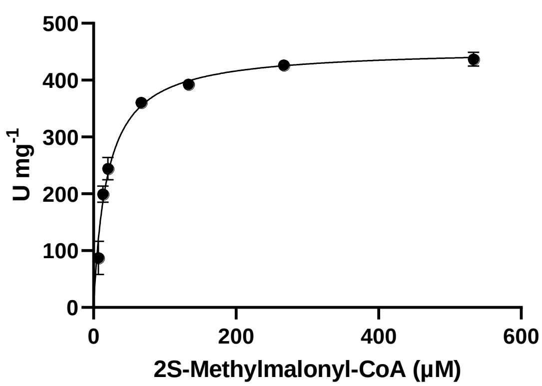
Supplemental Figure 1. Ccr Kinetics



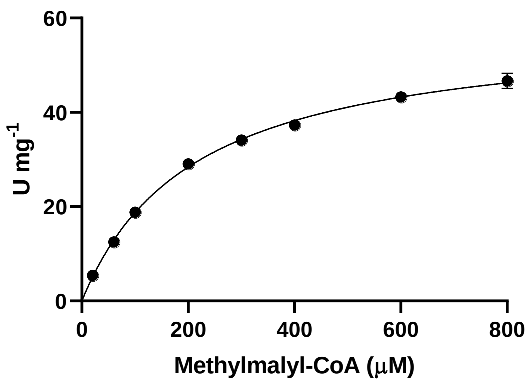
Supplemental Figure 2. Epi Activity



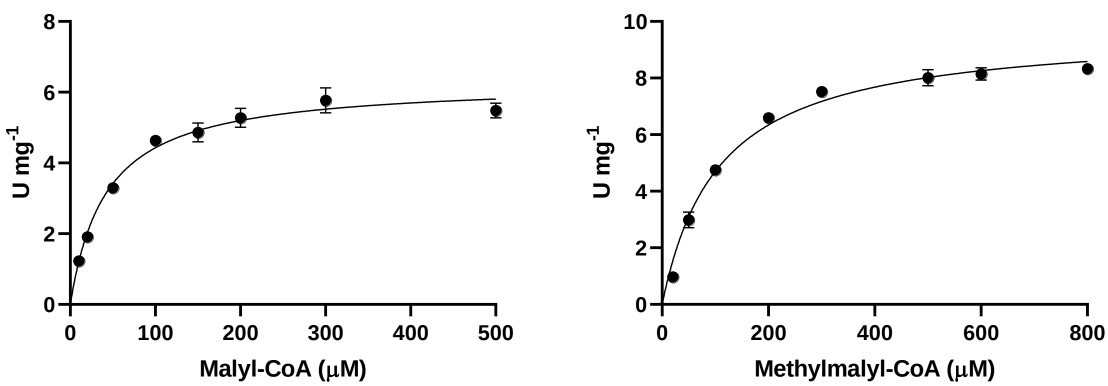
Supplemental Figure 3. Mcm Kinetics



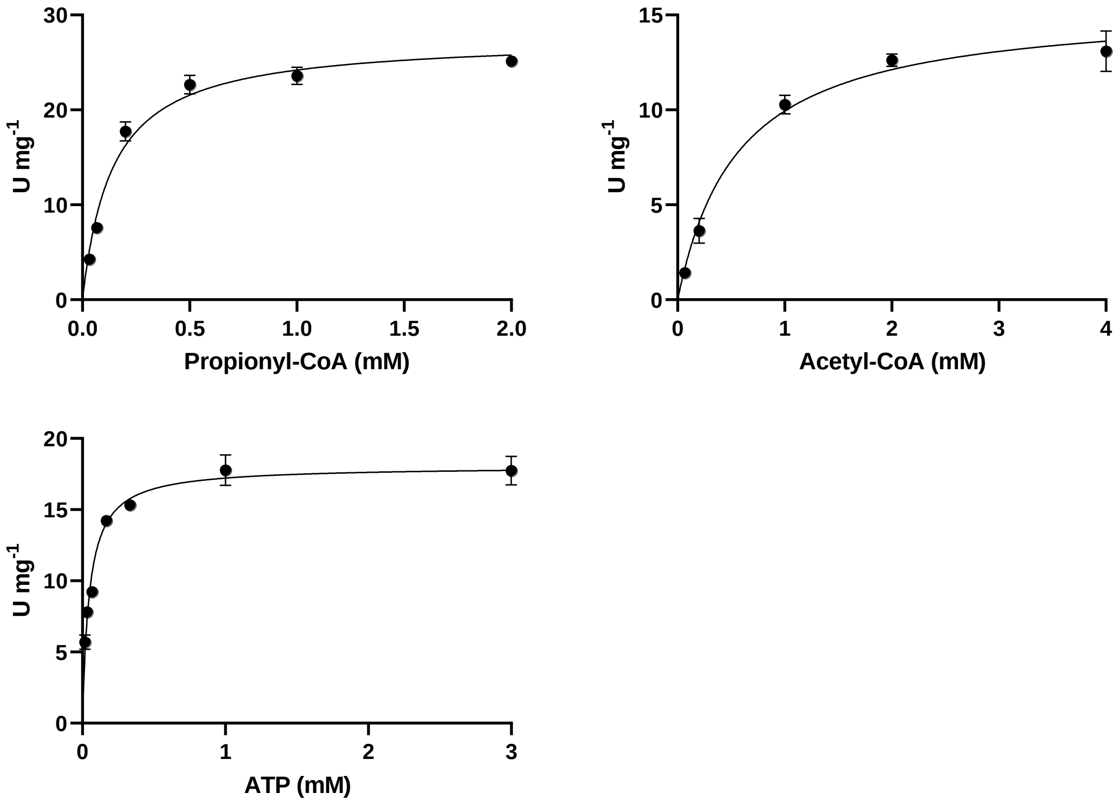
Supplemental Figure 4. Mch Kinetics



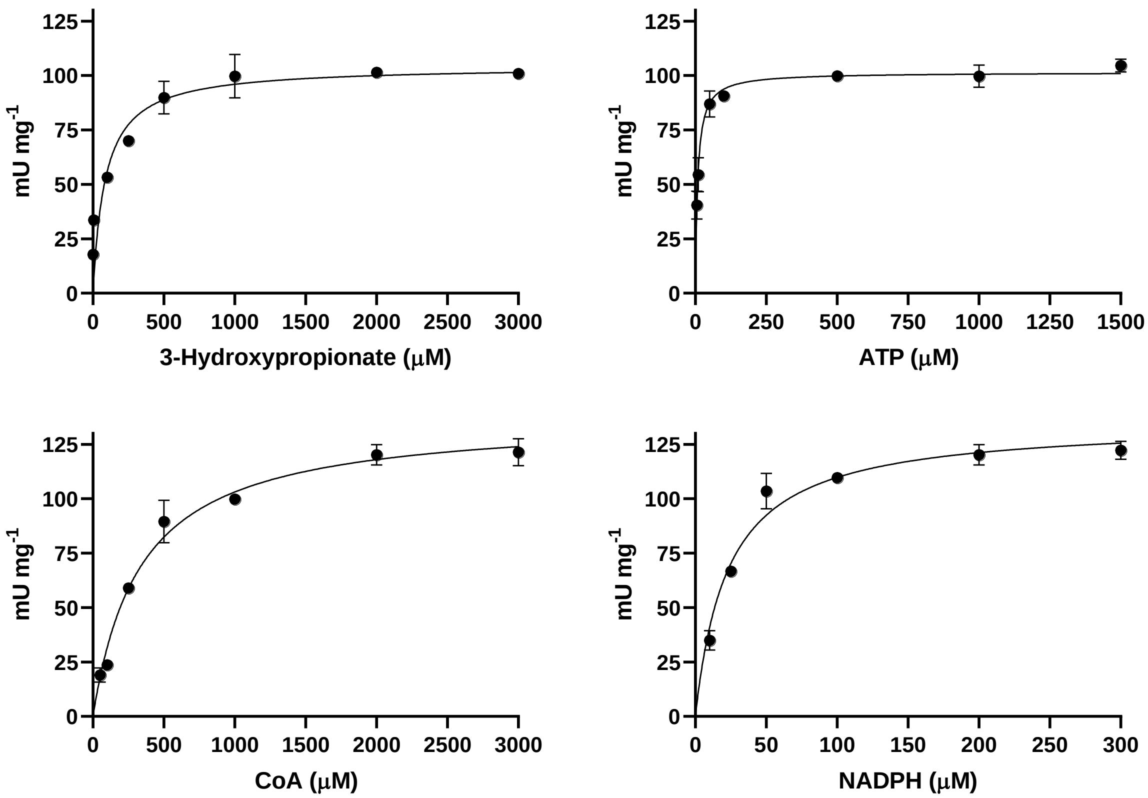
Supplemental Figure 5. Mcl Kinetics



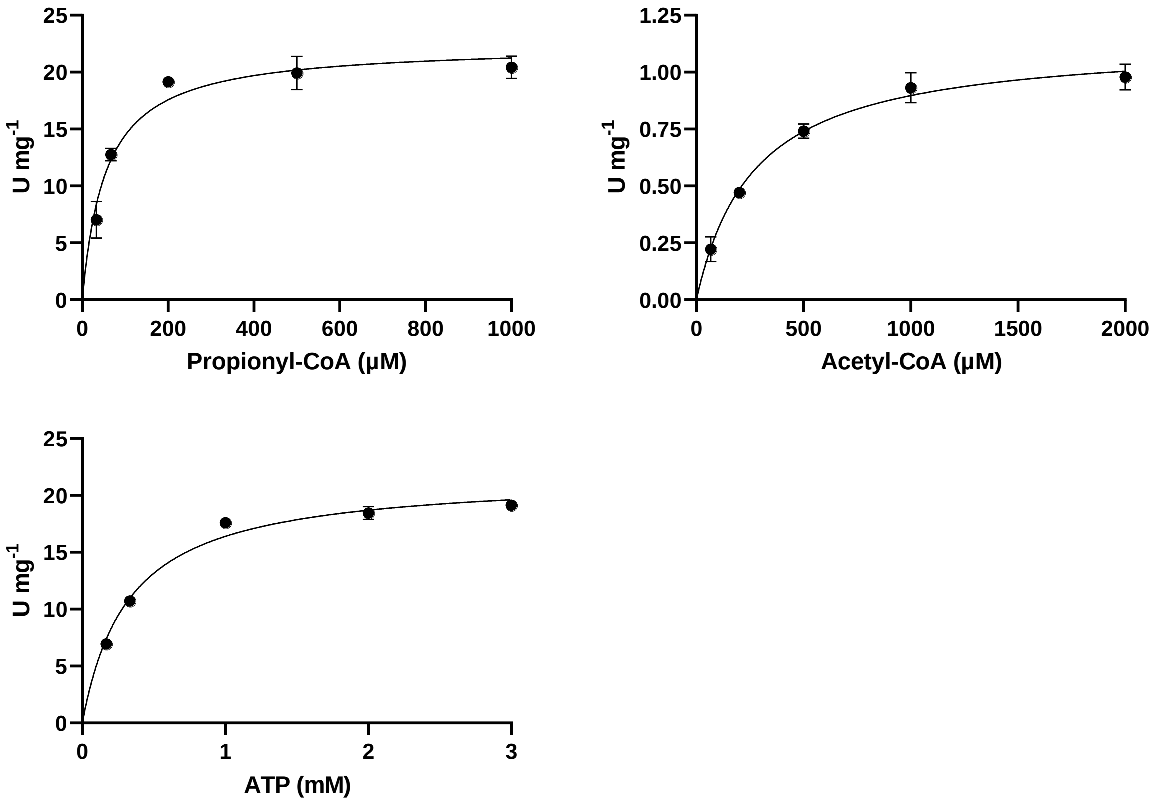
Supplemental Figure 6. $Pcc_{(D407I)}$ Kinetics



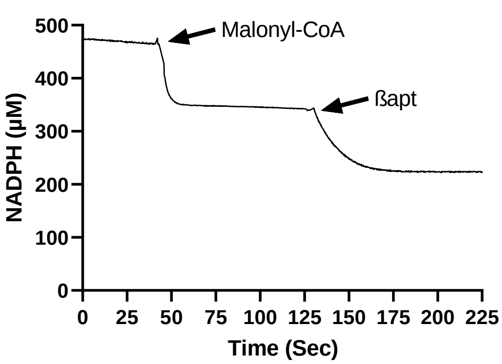
Supplemental Figure 7. Pcs Kinetics



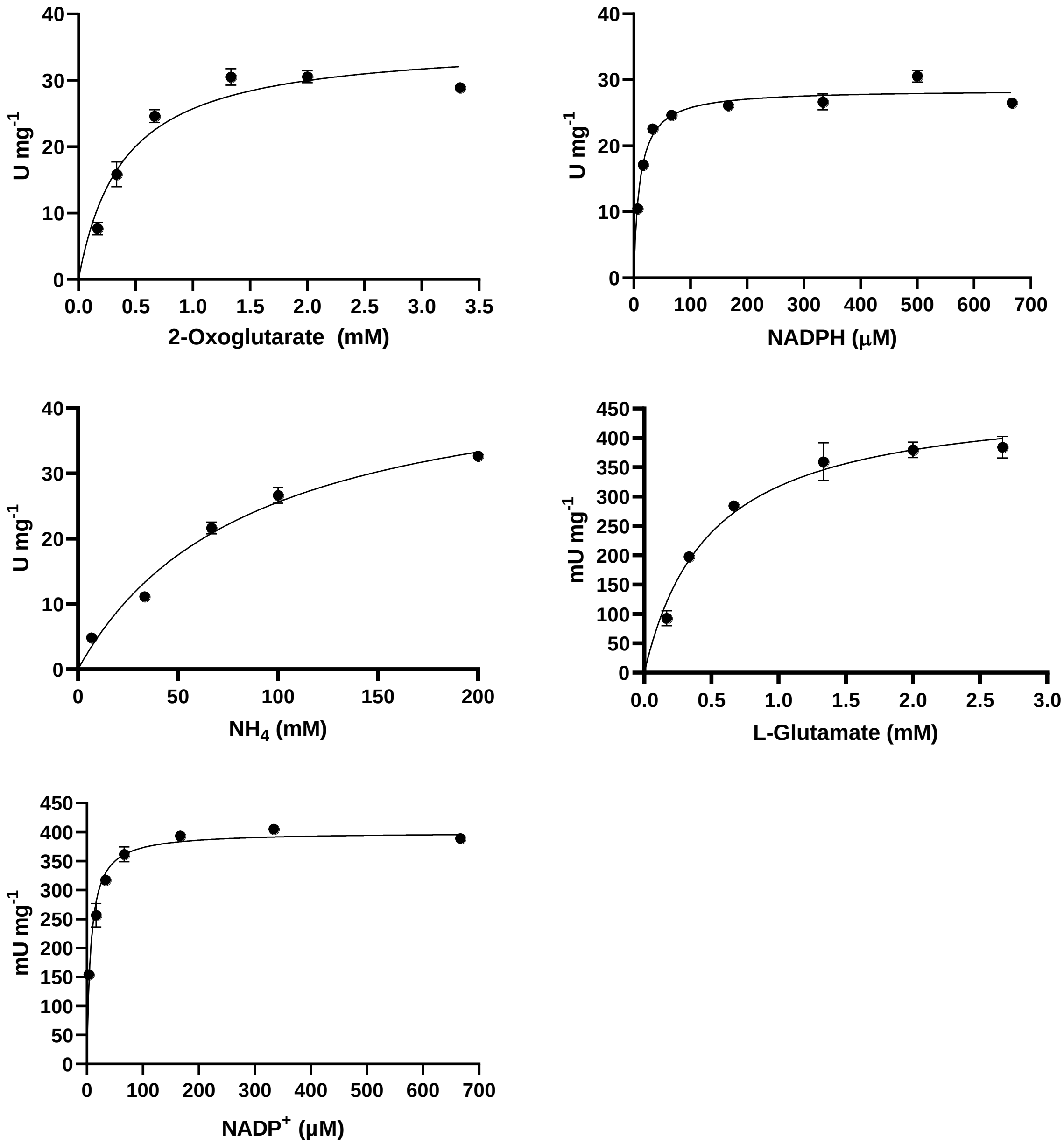
Supplemental Figure 8. Pcc Kinetics



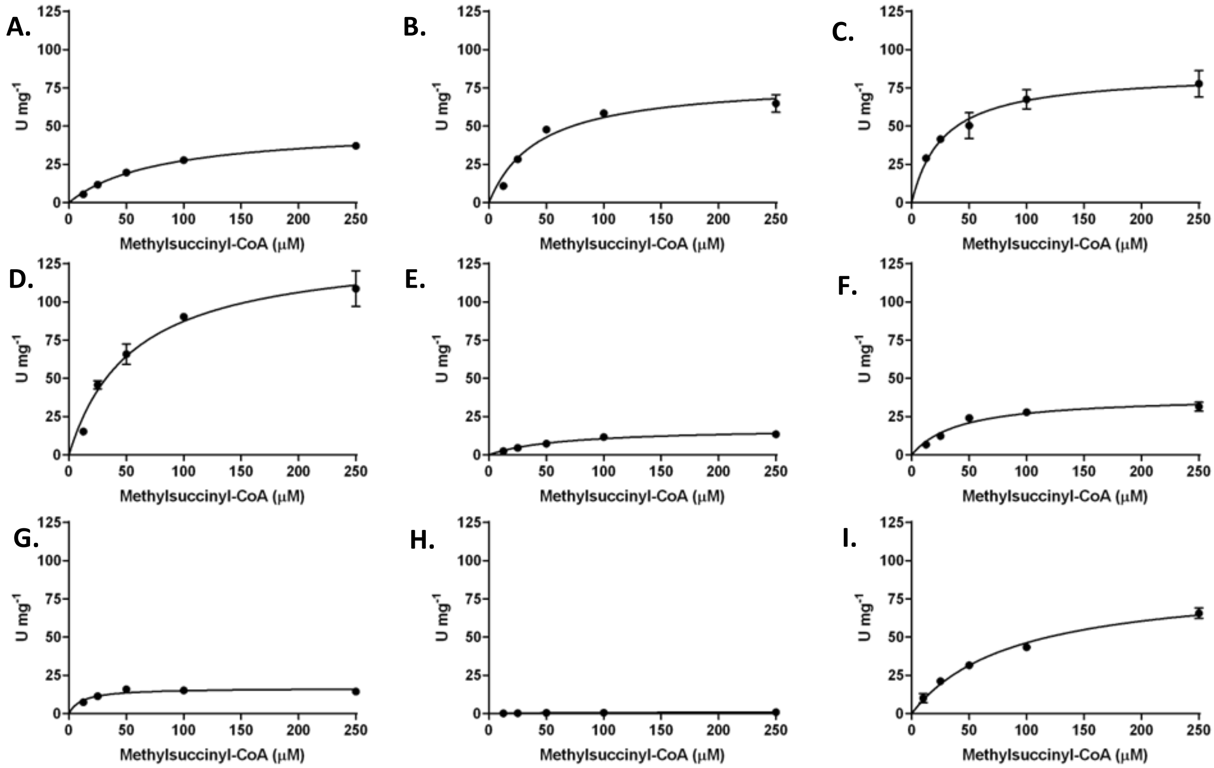
Supplemental Figure 9. βapt Activity



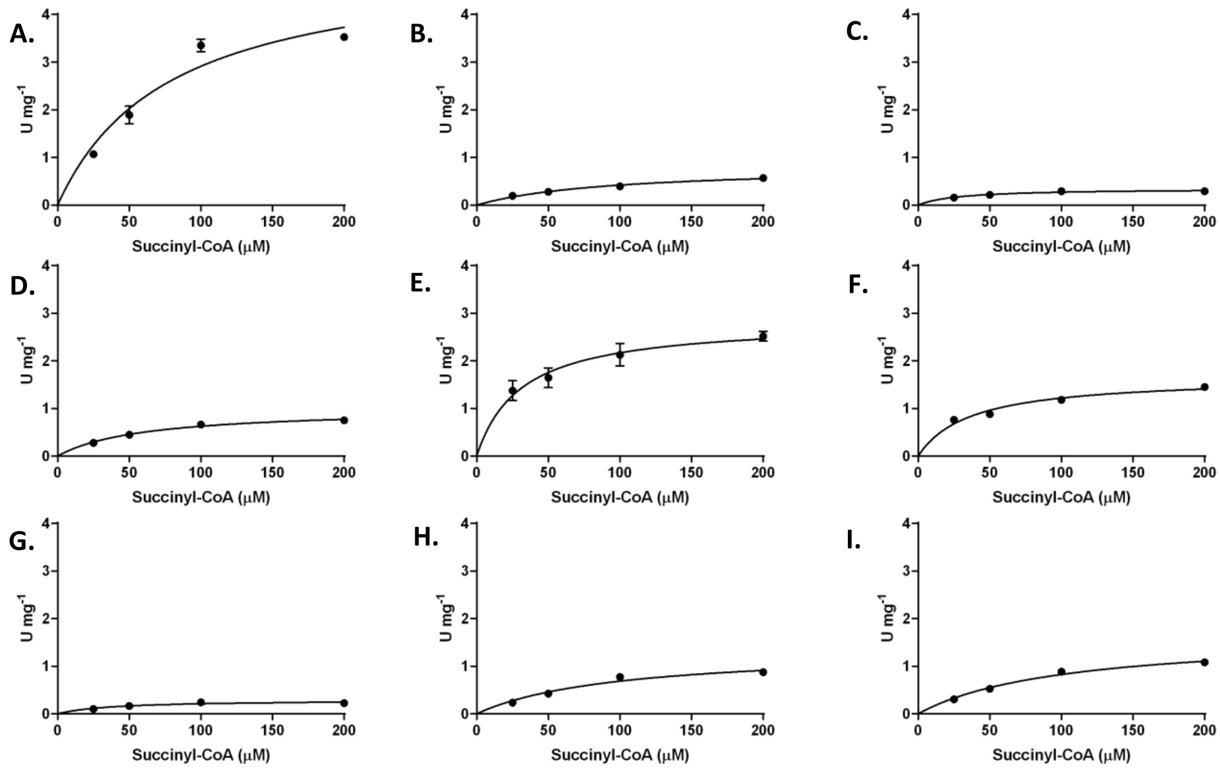
Supplemental Figure 10. Gdh Kinetics



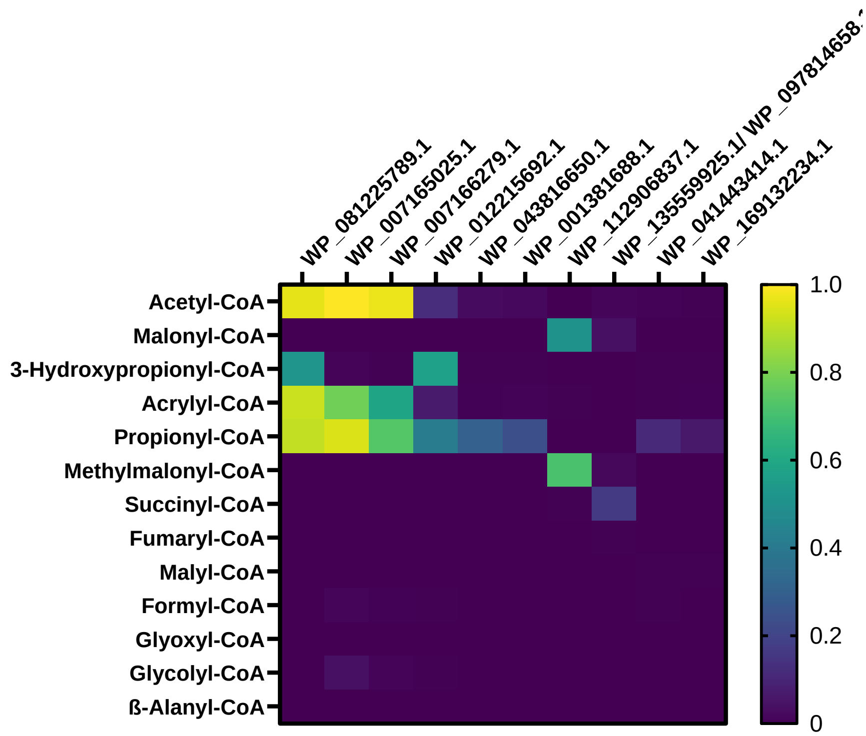
Supplemental Figure 11. Mcd kinetics on methylsuccinyl-CoA **A.** PmMcd **B.** CsMcd **C.** SaMcd **D.** WmMcd **E.** CvMcd **F.** ElMcd **G.** FsMcd **H.** PdMcd **I.** HrMcd.



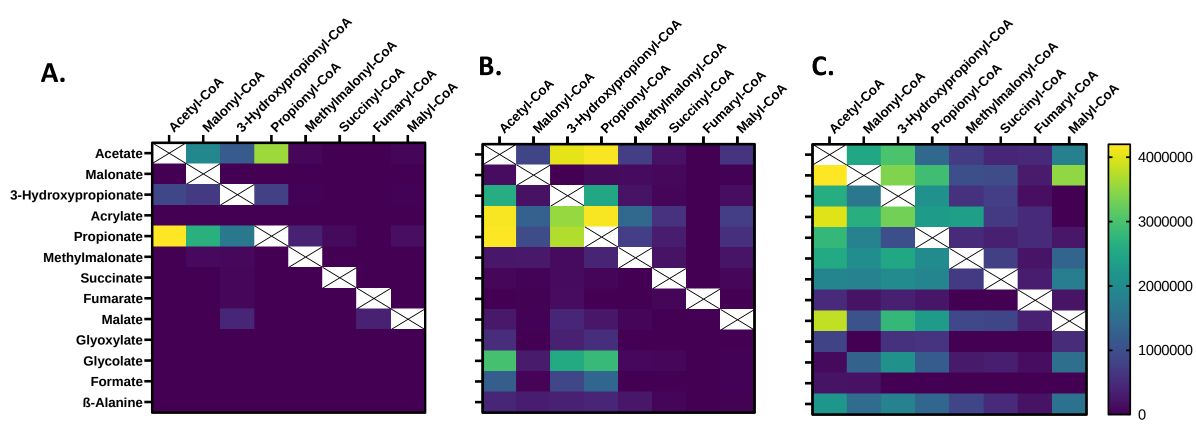
Supplemental Figure 12. Mcd kinetics on succinyl-CoA **A.** PmMcd **B.** CsMcd **C.** SaMcd **D.** WmMcd **E.** CvMcd **F.** ElMcd **G.** FsMcd **H.** PdMcd **I.** HrMcd.



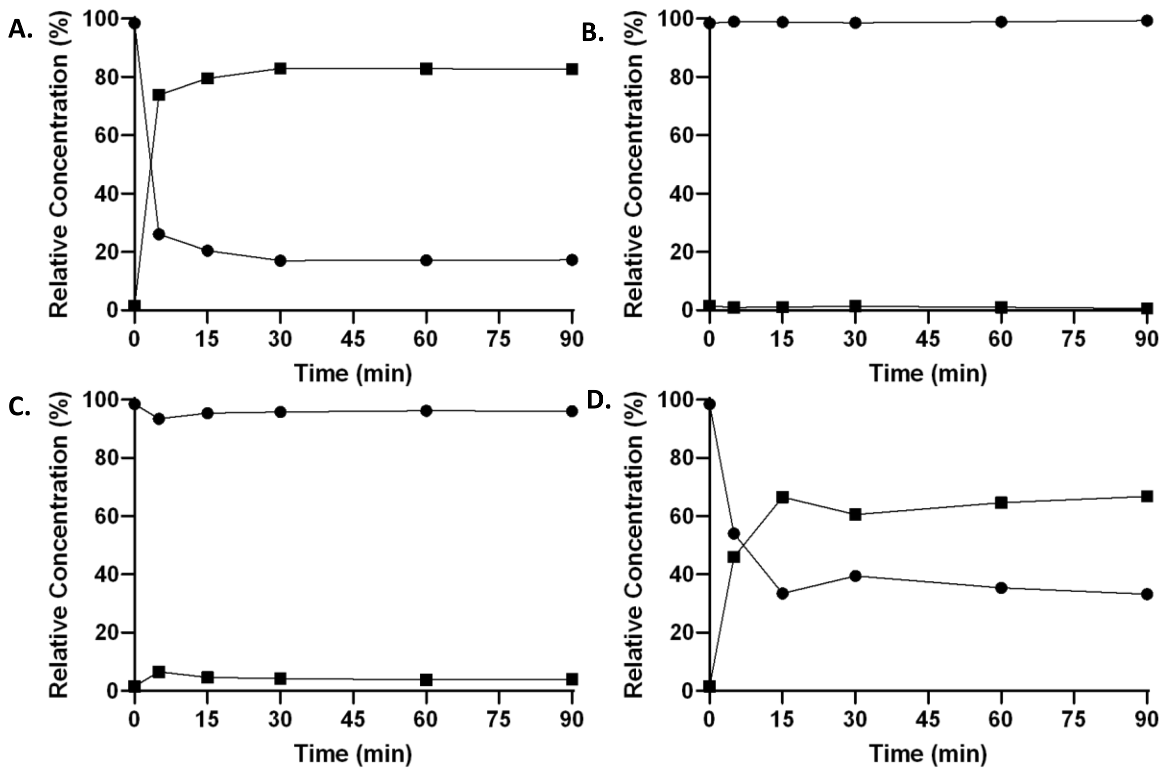
Supplemental Figure 13. Acyl-CoA synthetase activity screen. WP_081225789.1 = propioyl-CoA synthetase from *C. necator*. WP_007165025.1 and WP_007166279.1 = acetyl-CoA synthetase from *Erythrobacter* sp, NAP1. WP_012215692.1 = 3-hydroxypropionyl-CoA from *Nitrosopumilus maritimus*. WP_043816650.1 = butyryl-CoA synthetase from *Deinococcus maricopensis*. WP_001381688.1 = propioyl-CoA synthetase from *Escherichia coli*. WP_112906837.1 = malonyl-CoA synthetase from *Rhizobium leguminosarum*. WP_135559925.1/WP_097814658.1 = succinyl-CoA synthetase from *Escherichia coli*. WP_041443414.1 = phenylacetyl-CoA synthetase from *Thermus thermophilus*. WP_169132234.1 = phenylacetyl-CoA synthetase from *Aromatoleum evansii*.



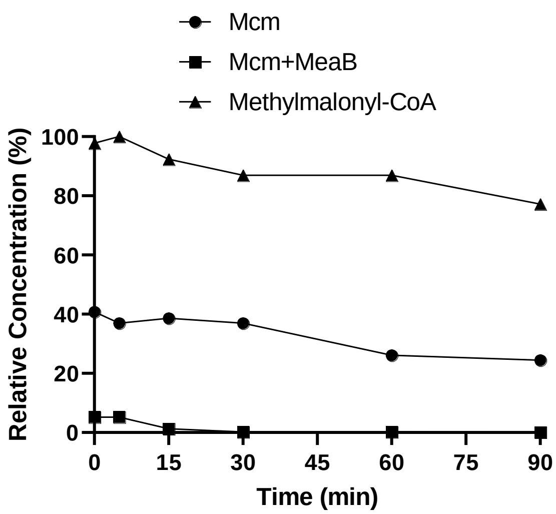
Supplemental Figure 14. Acyl-CoA transferase screen. **A.** Act, **B.** Pct, **C.** βct.



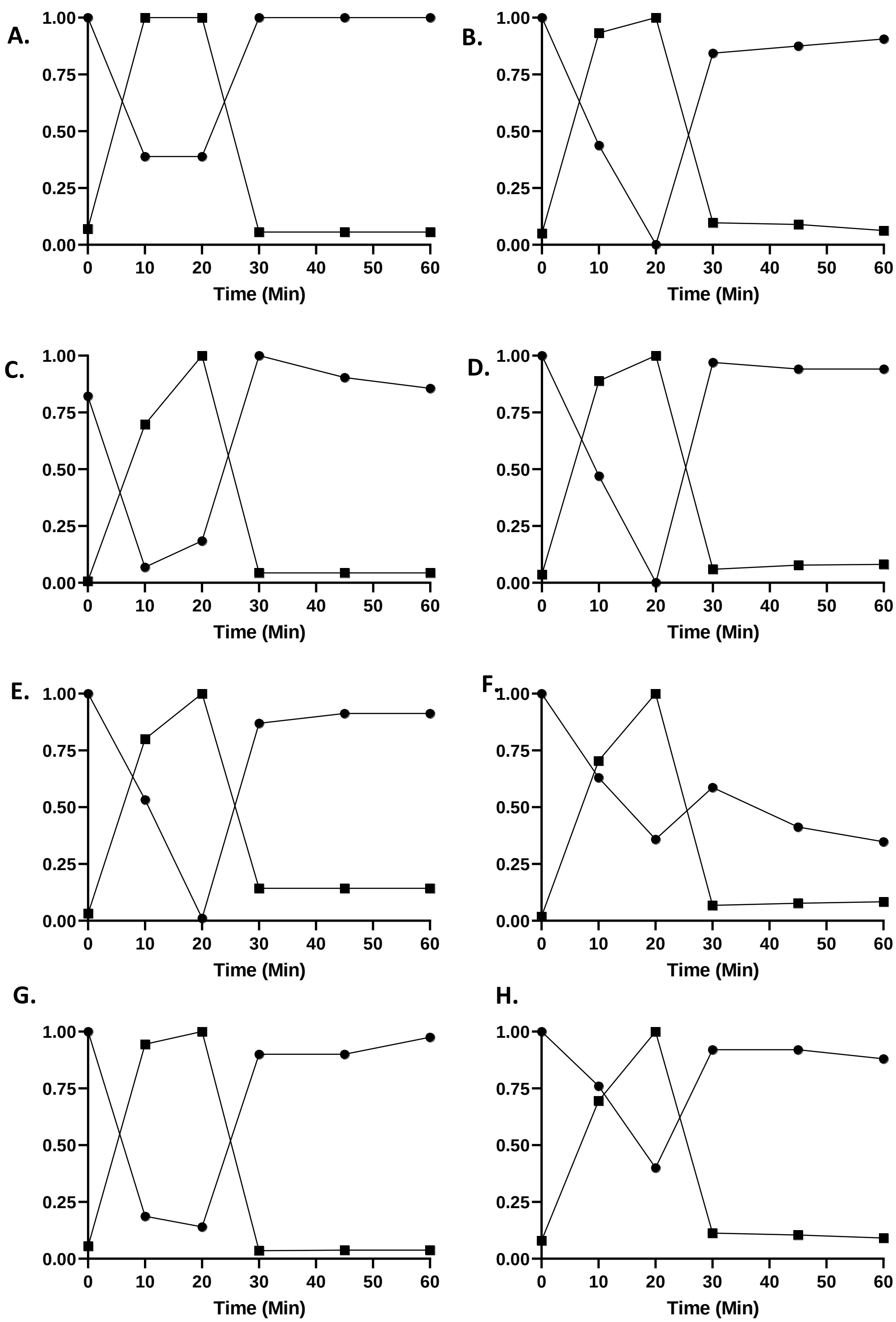
Supplemental Figure 15. Effect of Etf/EtfQO on Mcd activity. Succinyl-CoA (circles) is oxidized to fumaryl-CoA (squares) by Mcd in the presence of **A.** ferrocenium, **B.** O_2, **C.** Etf, **D.** Etf and EtfQO.



Supplemental Figure 16. Effect of MeaB on extending Mcm lifespan. Methylmalonyl-CoA was incubated alone (triangles), with Mcm (circles), or with Mcm and MeaB (squares) for 90 minutes. Samples were removed and incubated with Scr and NADPH to remove succinyl-CoA. When Mcm is inactivated, succinyl-CoA is removed, and the pool of methylmalonyl-CoA is maintained. When it remains active, succinyl-CoA is regenerated as it is consumed by Scr.

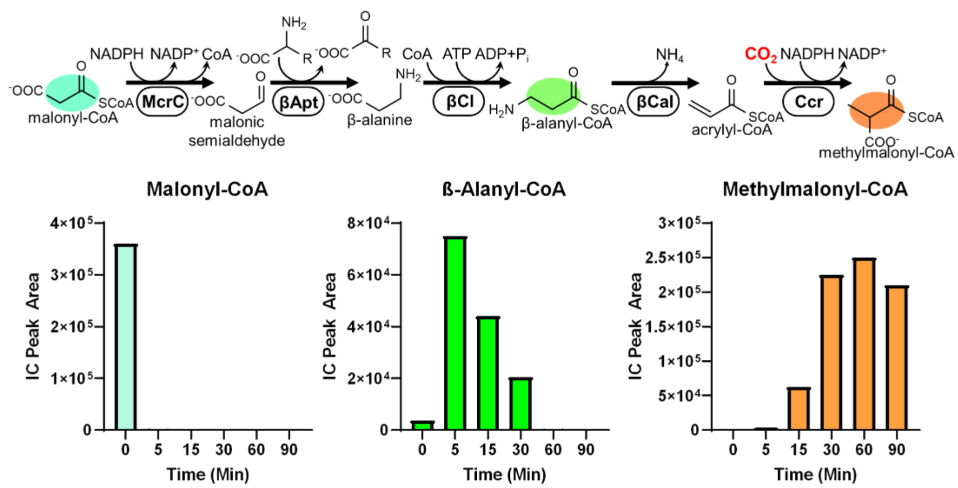


Supplemental Figure 17. Activity of CoAE on **A.** acetyl-CoA. **B.** malonyl-CoA. **C.** 3-hydroxypropionyl-CoA. **D.** propionyl-CoA. **E.** methylmalonyl-CoA. **F.** fumaryl-CoA. **G.** (S)-malyl-CoA. **H.** β-alanyl-CoA. Acyl-CoAs (circles) are dephosphorylated (squares) then phosphorylated by CoAE.

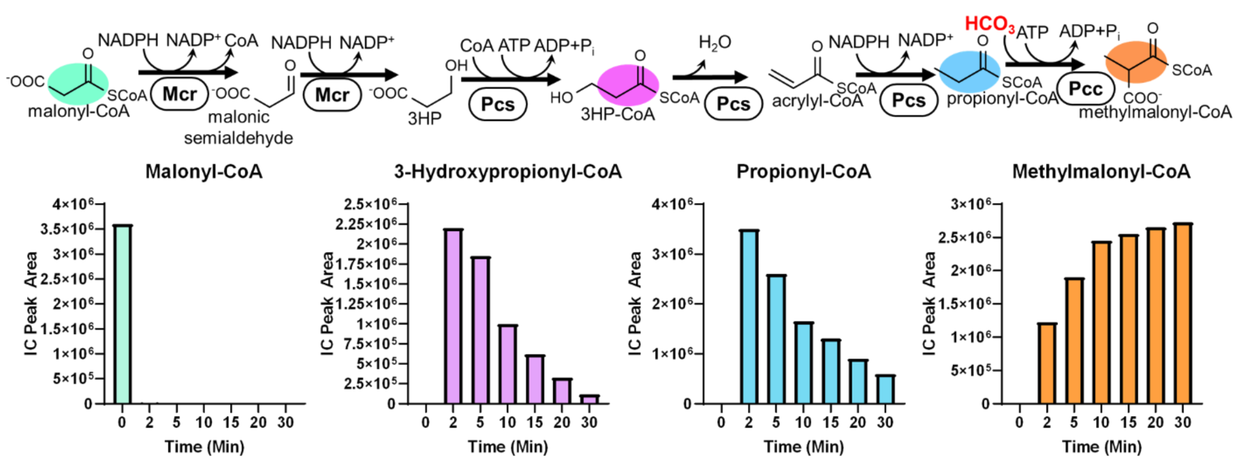


Supplemental Figure 18. Validation of the β-alanine route utilizing βapt and Adh. Values refer to relative concentrations of intermediates as determined by LCMS.

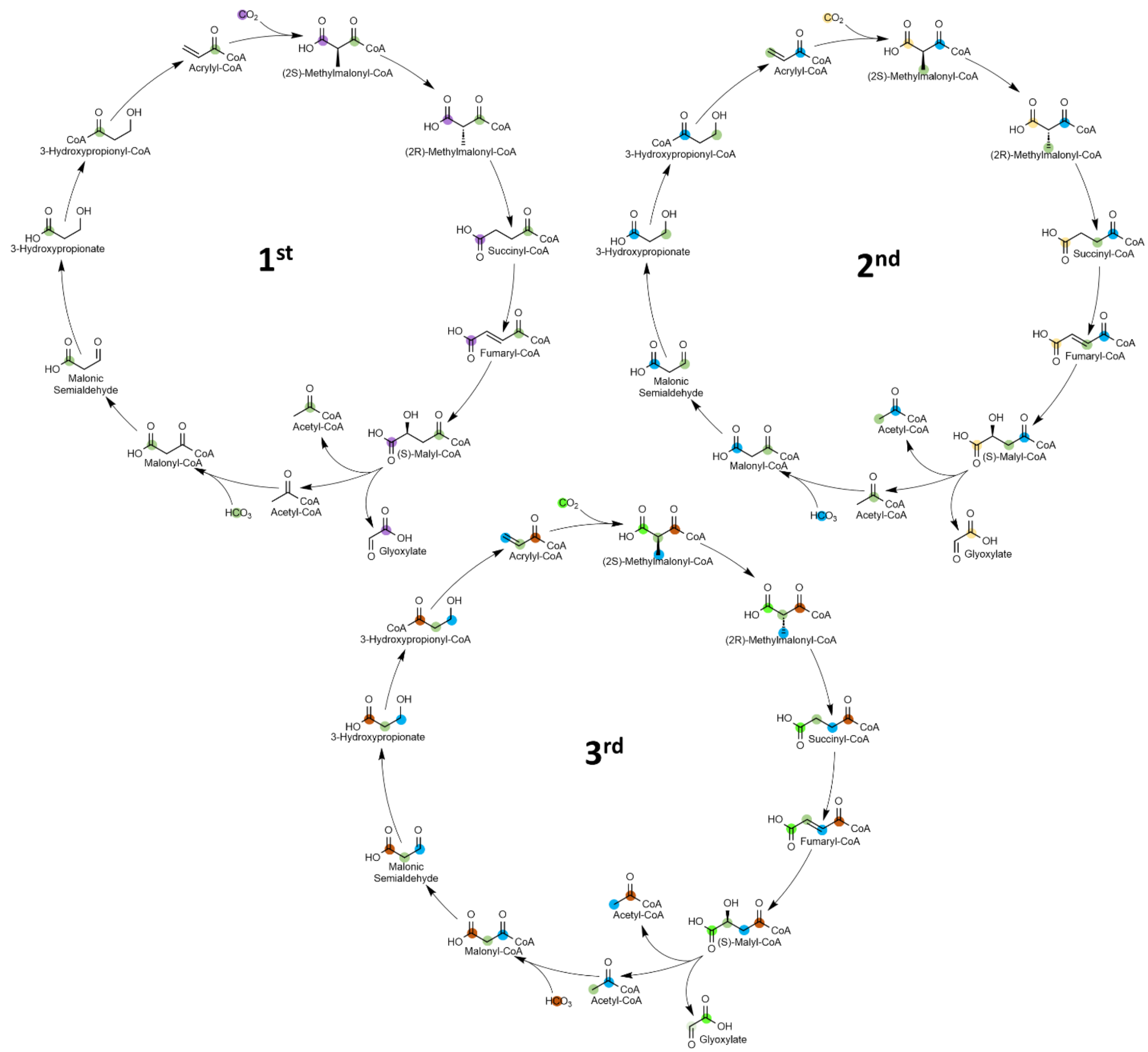
a



Supplemental Figure 19. Validation of the 3OH route utilizing Pcs and Pcc. Values refer to relative concentrations of intermediates as determined by LCMS.



Supplemental Figure 20. Expected ^{13}C-label incorporation of the HOPAC cycle from $^{13}CO_3^-$ and $^{13}CO_2$. The reaction is started with ^{12}C acetyl-CoA, and the first carboxylation incorporates a single ^{13}C from $^{13}CO_3^-$ labelled in the first cycle with a green circle. A second 13C is incorporated in the reductive carboxylation step, this time from $^{13}CO_2$, labelled with a purple circle, provided by carbonic anhydrase. At the completion of the first turn of the cycle, malyl-CoA is cleaved into glyoxylate containing the reductively carboxylated ^{13}C, and acetyl-CoA containing the originally fixed ^{13}C. The second turn of the cycle begins with this singly labelled acetyl-CoA, and the process is repeated. The product of the second turn of the cycle is twice labelled acetyl-CoA, at the completion of the third turn, the acetyl-CoA is again twice labelled making any further cycling indiscriminable. The process is identical when Pcs or β-alanine variants are used, but the method loses utility in the free acid path due to the symmetry of fumarate which causes two pools of malate to be formed in the second turn of the cycle, labelled at the 1,2,4 or 1,3,4 carbons.

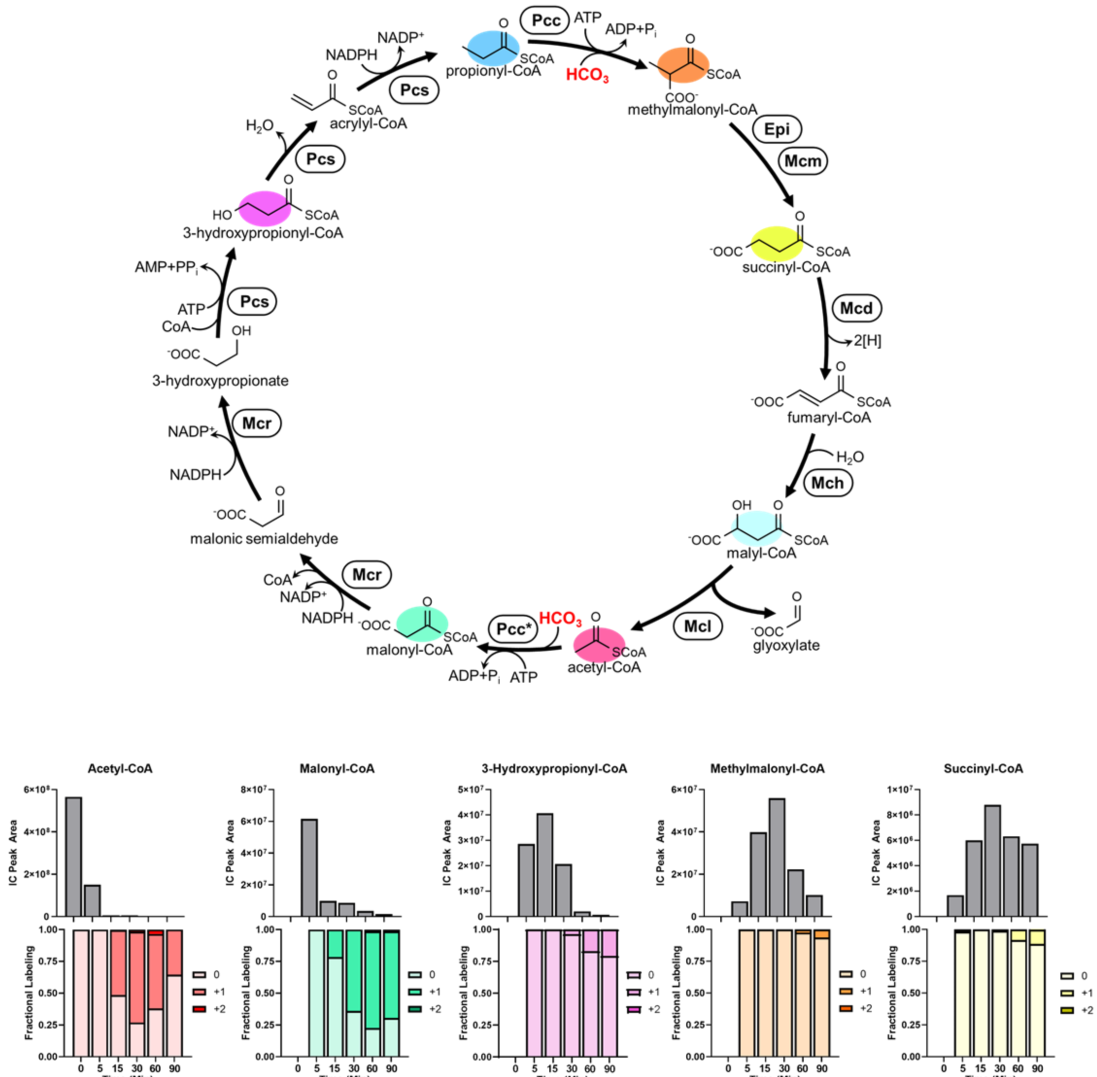


Supplemental Figure 21. HOPACPcs/Pcc (non-reductive carboxylation variant). **A.** Construction of the HOPACPcs/Pcc, CoA ligation to 3-hydroxypropionate, 3-hydroxypropionyl-CoA dehydration, and acrylyl-CoA reduction are all performed by the multicatalytic enzyme Pcs. The generation of propionyl-CoA removes the ability to reductively carboxylate with Ccr, so Pcc is added to complete carboxylation in an ATP-dependent manner resulting in an additional net cost of 1 ATP per cycle. **B.** Flux through the cycle over 90 minutes, values given are ion counts derived by LCMS. **C.** Fractional labelling of intermediates, colors correspond to (A.), rationale for labelling can be found in Fig. S20. Legend numbers are normalized to cycle completion, 0 is prior to completing a cycle, +1 is after successful completion of a cycle, +2 is after two.

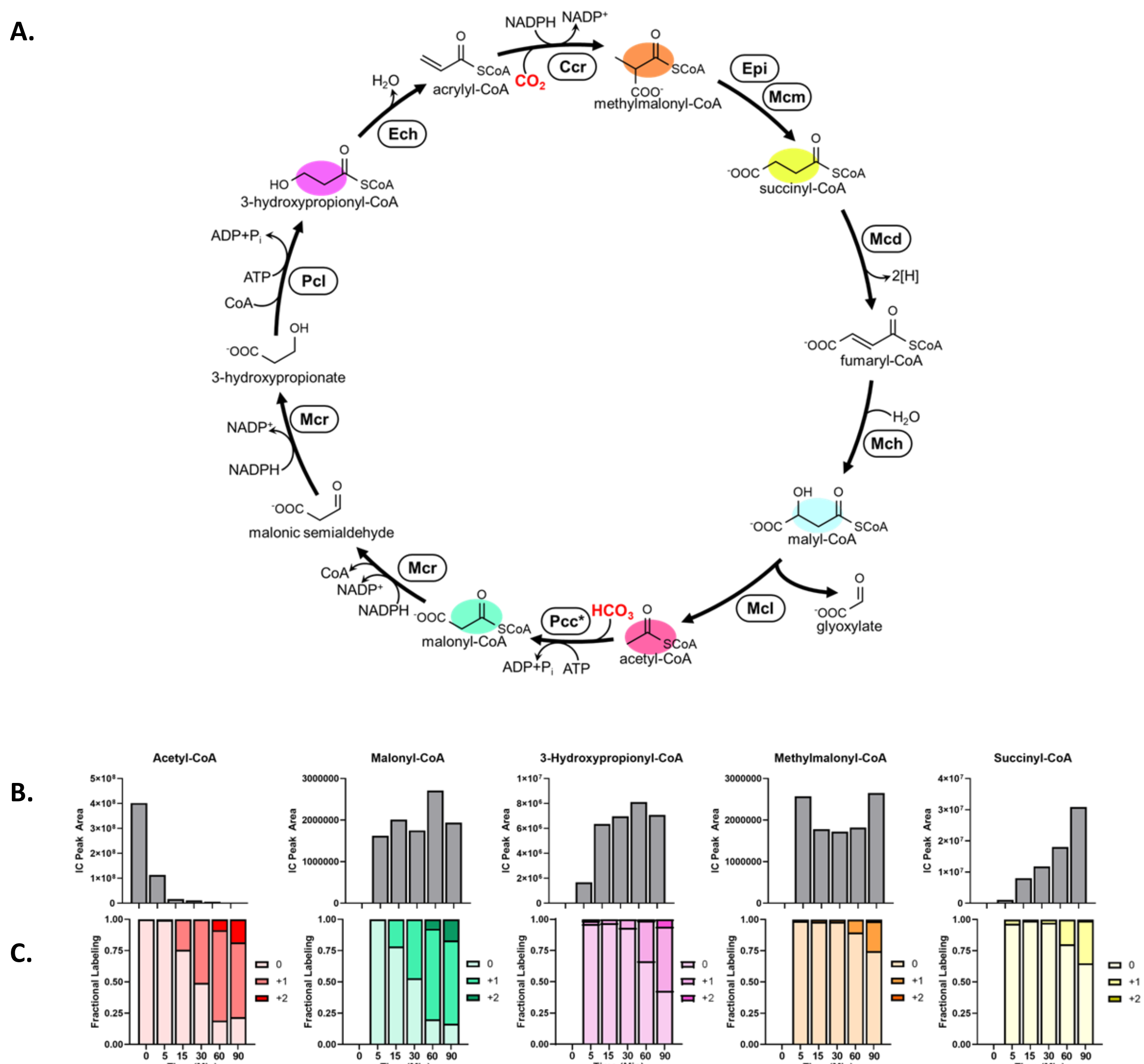
A.

B.

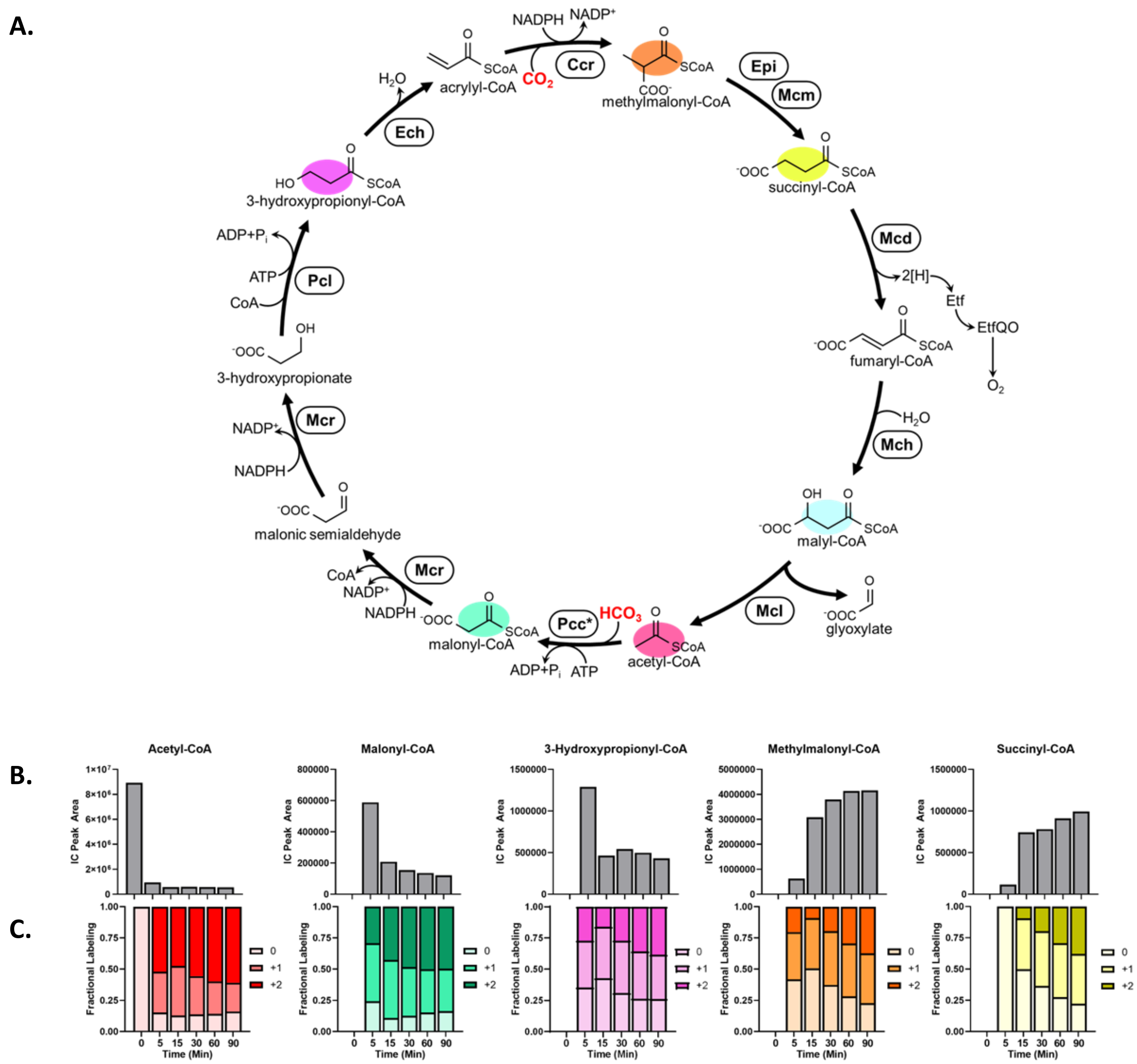
C.



Supplemental Figure 22. HOPACCcr 1.0 (reductive carboxylation variant). **A.** Construction of the HOPACCcr 1.0, CoA ligation to 3-hydroxypropionate is performed by Pcl and 3-hydroxypropionyl-CoA dehydration is performed by Ech allowing for the reductive carboxylation of acrylyl-CoA by Ccr. Bypassing the ATP-dependent carboxylation of propionyl-CoA resulting in a net savings of 1 ATP per cycle. **B.** Flux through the cycle over 90 minutes, values given are ion counts derived by LCMS. **C.** Fractional labelling of intermediates, colors correspond to (A.), rationale for labelling can be found in Fig. S20. Legend numbers are normalized to cycle completion, 0 is prior to completing a cycle, +1 is after successful completion of a cycle, +2 is after two.

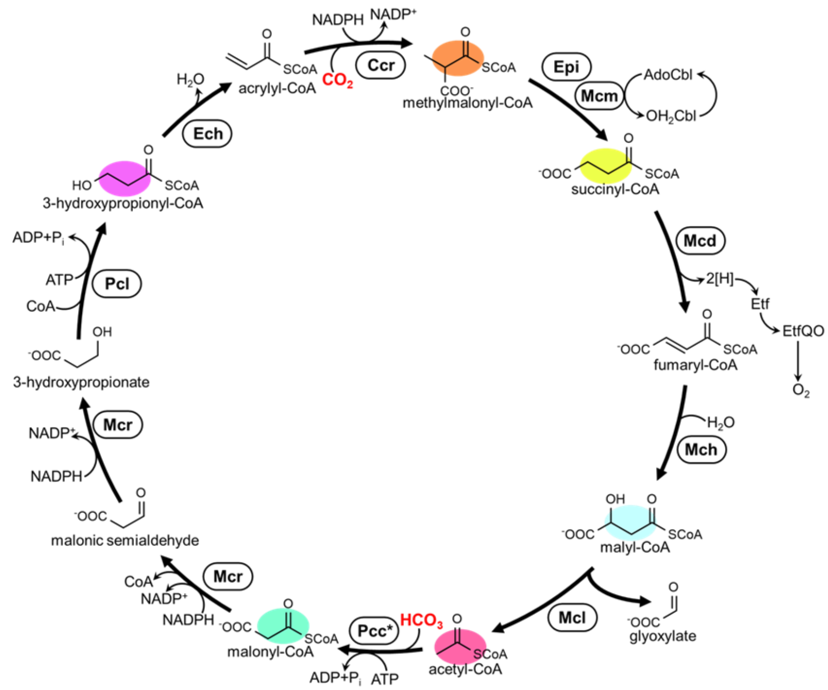


Supplemental Figure 23. HOPACCcr 2.0 (addition of the electron carriers). **A.** Construction of the HOPACCcr 2.0, the minimal oxidase activity of Mcd causes a significant bottleneck at succinyl-CoA. The addition of Etf, and solubilized EtfQO which can utilize O_2 as an electron acceptor allow for the relief of this bottleneck. **B.** Flux through the cycle over 90 minutes, values given are ion counts derived by LCMS. **C.** Fractional labelling of intermediates, colors correspond to (A.), rationale for labelling can be found in Fig. S20. Legend numbers are normalized to cycle completion, 0 is prior to completing a cycle, +1 is after successful completion of a cycle, +2 is after two.

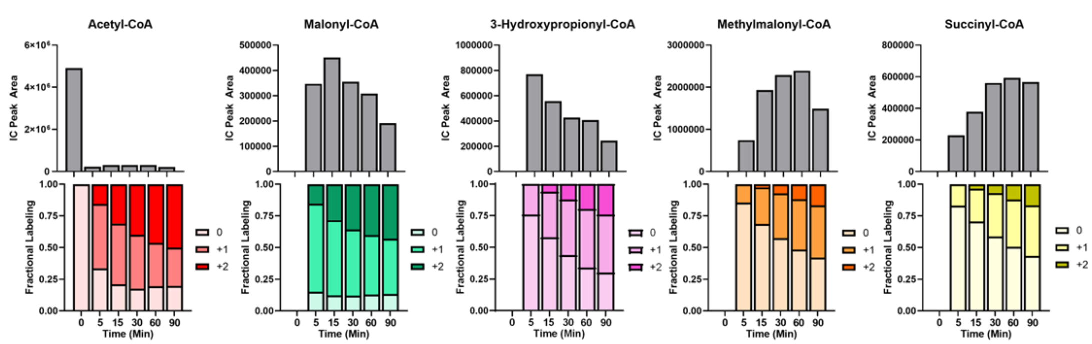


Supplemental Figure 24. HOPACCcr 3.1 (Mcm recovery). **A.** Construction of the HOPACCcr 3.0, Mcm suicide inactivation causes a significant bottleneck at methylmalonyl-CoA. The addition of the native Mcm chaperone MeaB extends the life of the enzyme and modestly improves cycling, however the periodic supplementation of fresh Mcm allows for the relief of this bottleneck. **B.** Flux through the cycle over 90 minutes, values given are ion counts derived by LCMS. **C.** Fractional labelling of intermediates, colors correspond to (A.), rationale for labelling can be found in Fig. S20. Legend numbers are normalized to cycle completion, 0 is prior to completing a cycle, +1 is after successful completion of a cycle, +2 is after two.

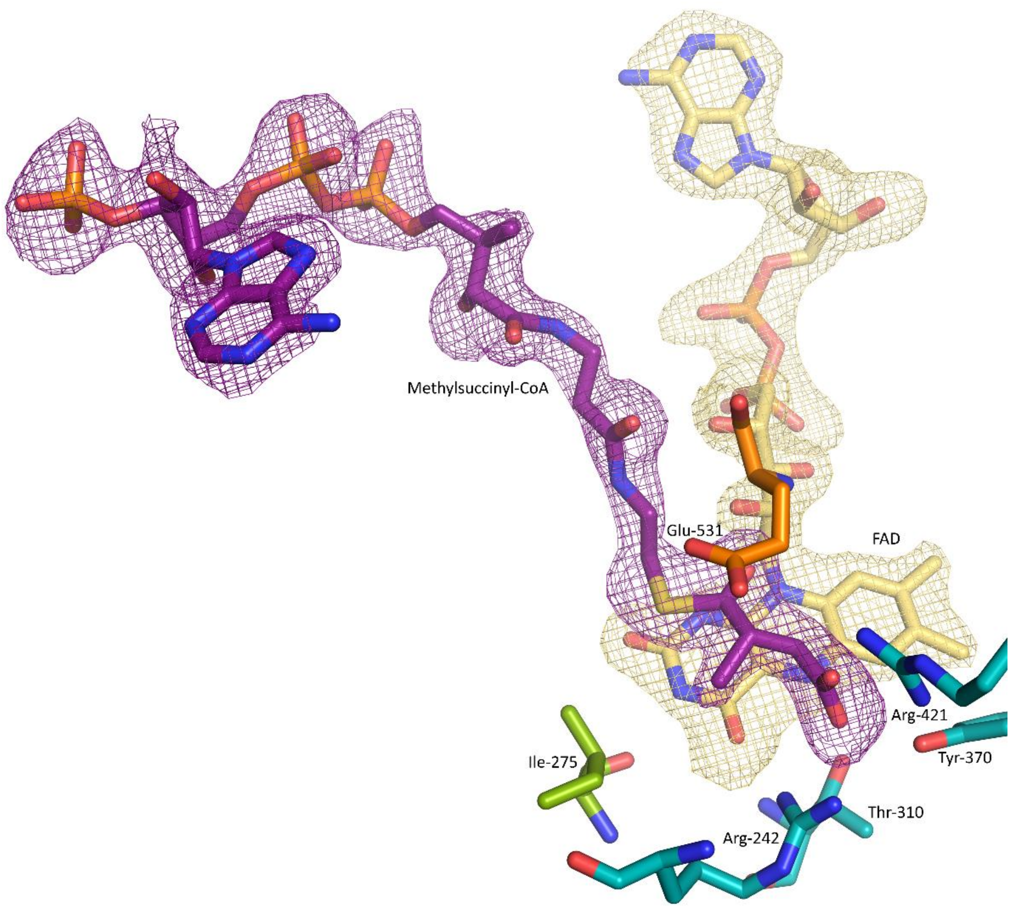
A.



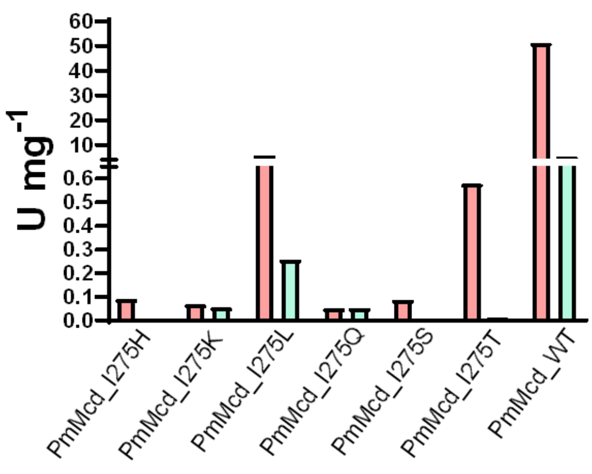
A.



Supplemental Figure 25. Simulated annealing omit-maps for bound ligands in PmMcd (PDB: 8CIW). Depicted are Fo-Fc electron density maps at a σ of 2.5 for FAD (yellow) and (2*S*)-methylsuccinyl-CoA (purple).



Supplemental Figure 26. Mutant Mcd activities on methylsuccinyl-CoA (red) and succinyl-CoA (green).



Data S1: HOPAC METIS Optimization Setup.

Amino Acid Sequences

Methylorubrum extorquens Ccr
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EYHIAGSDASGIVWKVGAKVKRWKVGDEVIVHCNQDDGDDEECNGGDPMFSPTQRIWGYETGDGSFAQFCRVQSRQLMARPKHLTWEEAACYTLTLA
TAYRMLFGHAPHTVRPGQNVLIWGASGGLGVFGVQLCAASGANAIAVISDESKRDYVMSLGAKGVINRKDFDCWGQLPTVNSPEYNTWLKEARKFGK
AIWDITGKGNDVDIVFEHPGEATFPVSTLVAKRGGMIVFCAGTTGFNITFDARYVWMRQKRIQGSHFAHLKQASAANQFVMDRRVDPCMSEVFPWDK
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Cereibacter sphaeroides Epi
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IHHICYEVEDILAARDRLKEAGARVLGSGEPKIGAHGKPVLFLHPKDFNGCLVELEQV-

Cereibacter sphaeroides Mcm
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DDFAGRLSFFFAIGMNFFMEAAKLRAARLLWHRIMSEFAPKKPGSLMLRTHCQTSGVSLQEQDPYNNVIRTAYEAMSAALGGTQSLHTNALDEAIAL
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LAKEDPIEILDIDNVAVRDAQIARLEKMRATRDEAACQAALDELTRRAAEGGNLLEAAVDASRARASVGEISMAMEKVFGRHRAEVKTLSGVYGAAY
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LKEKGAGEILVICGGVIPQQDYDFLQQAGVKAIFGPGTNIPSAAKHILDLIREARS-

Cereibacter sphaeroides MeaB
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Pseudomonas migulae Mcd
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AEHIHREDLIIPDSLLAALRDMGVFGLSIPERYGGSAPDDQEDPLTMIVVTEALSQASLAAAGSLITRPEILSRALLSGGTESQKQHWLARLAVGDP
LCAIAITEPDYGSDVAGLTLRGTPCEGGWRLNGAKTWCTFAGKAGVLMVVTRTNPDKSLGHRGLSLLLAEKPSYDGHEFDFRQPGGGSLTGRAIPTI
GYRGMHSFDLSFEDFFVPDGNVIGEAQGLGKGFYHTMAGMTGGRMQTAGRASGVMRAALLAGLRYATERKVFGSPLLDYPLTGAKLTKMAARYVASR
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Pseudomonas migulae Etf
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INKDEEAPIFQVADYGLVADLFEAIPEFEKLV-

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Pseudomonas migulae EtfQO
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Cereibacter sphaeroides Mch
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Cereibacter sphaeroides Mcl
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Methylorubrum extorquens Pcc(D407I)
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Chloroflexus aurantiacus Mcr
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Cupriavidus necator Pcl
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Pseudomonas aeruginosa Ech
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Gluconobacter oxydans Grd
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Escherichia coli Scs
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DAAQQVVAAVEGK-

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Chloroflexus aurantiacus Smt
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LGMCDEQINDLRSKRVI-

Mus musculus Sch
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Escherichia coli Sdh
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IEYMCKTGPEAILELEHMGLPFSRLDDGRIYQRPFGGQSKNFGGEQAARTAAAADRTGHALLHTLYQQNLKNHTTIFSEWYALDLVKNQDGAVVGCT
ALCIETGEVVYFKARATVLATGGAGRIYQSTTNAHINTGDGVGMAIRAGVPVQDMEMWQFHPTGIAGAGVLVTEGCRGEGGYLLNKHGERFMERYAP
NAKDLAGRDVVARSIMIEIREGRGCDGPWGPHAKLKLDHLGKEVLESRLPGILELSRTFAHVDPVKEPIPVIPTCHYMMGGIPTKVTGQALTVNEKG
EDVVVPGLFAVGEIACVSVHGANRLGGNSLLDLVVFGRAAGLHLQESIAEQGALRDASESDVEASLDRLNRWNNNRNGEDPVAIRKALQECMQHNFS
VFREGDAMAKGLEQLKVIRERLKNARLDDTSSEFNTQRVECLELDNLMETAYATAVSANFRTESRGAHSRFDFPDRDDENWLCHSLYLPESESMTRR
SVNMEPKLRPAFPPKIRTY-

MRLEFSIYRYNPDVDDAPRMQDYTLEADEGRDMMLLDALIQLKEKDPSLSFRRSCREGVCGSDGLNMNGKNGLACITPISALNQPGKKIVIRPLPGL
PVIRDLVVDMGQFYAQYEKIKPYLLNNGQNPPAREHLQMPEQREKLDGLYECILCACCSTSCPSFWWNPDKFIGPAGLLAAYRFLIDSRDTETDSRL
DGLSDAFSVFRCHSIMNCVSVCPKGLNPTRAIGHIKSMLLQRNA-

Escherichia coli Fuh
MRGSHHHHHHTDPALRANTVRSEKDSMGAIDVPADKLWGAQTQRSLEHFRISTEKMPTSLIHALALTKRAAAKVNEDLGLLSEEKASAIRQAADEVL
AGQHDDEFPLAIWQTGSGTQSNMNMNEVLANRASELLGGVRGMERKVHPNDDVNKSQSSNDVFPTAMHVAALLALRKQLIPQLKTLTQTLNEKSRAF
ADIVKIGRTHLQDATPLTLGQEISGWVAMLEHNLKISKQPASRNGLGLGGTTVGTGLIPSRRCASRTNRLESIPGARLFSPKILTWDRLAPSRPPPS
IKWRALKNTQNPRGVSPLQCCYPPEERYRREQHVAYFSLDEWK-

Methylorubrum extorquens Mcs
MDVHEYQAKELLASFGVAVPKGAVAFSPDQAVYAATELGGSFWAVKAQIHAGARGKAGGIKLCRTYNEVRDAARDLLGKRLVTLQTGPEGKPVQRVY
VETADPFERELYLGYVLDRKAERVRVIASQRGGMDIEEIAAKEPEALIQVVVEPAVGLQQFQAREIAFQLGLNIKQVSAAVKTIMNAYRAFRDCDGT
MLEINPLVVTKDDRVLALDAKMSFDDNALFRRRNIADMHDPSQGDPREAQAAEHNLSYIGLEGEIGCIVNGAGLAMATMDMIKHAGGEPANFLDVGG
GASPDRVATAFRLVLSDRNVKAILVNIFAGINRCDWVAEGVVKAAREVKIDVPLIVRLAGTNVDEGKKILAESGLDLITADTLTEAARKAVEACHGA
KH-

MKARRSSPRAGSTSSPPTPLRKPRARLSKPATAPSTDERGRNHAMSILIDEKTPILVQGITGDKGTFHAKEMIAYGSNVVGGVTPGKGGKTHCGVPV
FNTVKEAVEATGATTSITFVAPPFAADAIMEAADAGLKLVCSITDGIPAQDMMRVKRYLRRYPKEKRTMVVGPNCAGIISPGKSMLGIMPGHIYLPG
KVGVISRSGTLGYEAAAQMKELGIGISTSVGIGGDPINGSSFLDHLALFEQDPETEAVLMIGEIGGPQEAEASAWIKENFSKPVIGFVAGLTAPKGR
RMGHAGAIISATGDSAAEKAEIMRSYGLTVAPDPGSFGSTVADVLARAAAAALEHHHHHH-

Chloroflexus aurantiacus Mcr C-Term
MGSSHHHHHHSSGLVPRGSHMSSASVGWAESLIGLHLGKVALITGGSAGIGGQIGRLLALSGARVMLAARDRHKLEQMQAMIQSELAEVGYTDVEDR
VHIAPGCDVSSEAQLADLVERTLSAFGTVDYLINNAGIAGVEEMVIDMPVEGWRHTLFANLISNYSLMRKLAPLMKKQGSGYILNVSSYFGGEKDAA
IPYPNRADYAVSKAGQRAMAEVFARFLGPEIQINAIAPGPVEGDRLRGTGERPGLFARRARLILENKRLNELHAALIAAARTDERSMHELVELLLPN
DVAALEQNPAAPTALRELARRFRSEGDPAASSSSALLNRSIAAKLLARLHNGGYVLPADIFANLPNPPDPFFTRAQIDREARKVRDGIMGMLYLQRM
PTEFDVAMATVYYLADRNVSGETFHPSGGLRYERTPTGGELFGLPSPERLAELVGSTVYLIGEHLTEHLNLLARAYLERYGARQVVMIVETETGAET
MRRLLHDHVEAGRLMTIVAGDQIEAAIDQAITRYGRPGPVVCTPFRPLPTVPLVGRKDSDWSTVLSEAEFAELCEHQLTHHFRVARKIALSDGASLA
LVTPETTATSTTEQFALANFIKTTLHAFTATIGVESERTAQRILINQVDLTRRARAEEPRDPHERQQELERFIEAVLLVTAPLPPEADTRYAGRIHR
GRAITV-

Rhizobium hidalgonense βapt
MGSSHHHHHHSSGLVPRGSHMDQISKTNAPVLENFWMPFTANRQFKATPRLLAAADGMYYTDVDGNQVLDGTAGLWCCNAGHGRKKIARAVERQLST
LDYAPTFQMGHPIAFDFAAKLAANAPGGTDSKLDRVFFTGSGSESVDTALKIAIAYQRAIGQGTRTRIIGREKGYHGVGFGGISVGGLVNNRRVFPQ
IPADHMRHTLDVERNAFSKGLPAHGVELADDLERLVQLHGPETIAAVIVEPMSGSAGVVLPPKGYLEKLRATADKHGILLIFDEVITGFGRLGTPFA
VDYFGVVPDLVTTAKGLTNGAIPMGAVFASRKVYDGLMVGPENAIELFHGYTYSGHPVACAAGLATLEIYEEEGLLTRAAELAEYWQEGLHSLKGLP
HVVDIRNLGLVGAIELAPRAAAGTRAYDIFVDCFNKGLLIRVTGDIIALSPPLIIEKSQIDTIVSTIGDALKRAA-

Cereibacter sphaeroides Adh

MGSSHHHHHHSSGLVPRGSHMRIGCPKEIKPQEFRVGLTPHAAREAASHRHEVLVEAGAGAGAGFSDEDYRAAGARLVDTAEELFAAAELIVKVKEP
QPVERQRLREGQLLFTYLHLAPDPEQTRDLMASGVTAIAYETVTDTRGGLPLLAPMSEVAGRLAPQVGAWTLQKANGGRGVLLGGVPGVGPAKVVVI
GGGVVGTHAARIAAGMGADVTVLDRSLPRLRALDEAFGTLFRTSYASSGTTAELVTAADLVIGAVLIPGAAAPKLVSRAQLGTMKPGAAIVDVAIDQ
GGCFETSRPTTHQDPIYEVDGVMHYCVANMPGAVARTSTLALGNATMPFLLALADKGWKRACEEDPHLLAGLNTHAGHLTYYAVGRALEIDVLSPQL
ALKM-

Escherichia coli GabT
MRGSHHHHHHTDPALRANSNKELMQRRSQAIPRGVGQIHPIFADRAENCRVWDVEGREYLDFAGGIAVLNTGHLHPKVVAAVEAQLKKLSHTCFQVL
AYEPYLELCEIMNQKVPGDFAKKTLLVTTGSEAVENAVKIARAATKRSGTIAFSGAYHGRTHYTLALTGKVNPYSAGMGLMPGHVYRALYPCPLHGI
SEDDAIASIHRIFKNDAAPEDIAAIVIEPVQGEGGFYASSPAFMQRLRALCDEHGIMLIADEVQSGAGRTGTLFAMEQMGVAPDLTTFAKSIAGGFP
LAGVTGRAEVMDAVAPGGLGGTYAGNPIACVAALEVLKVFEQENLLQKANDLGQKLKDGLLAIAEKHPEIGDVRGLGAMIAIELFEDGDHNKPDAKL
TAEIVARARDKGLILLSCGPYYNVLRILVPLTIEDAQIRQGLEIISQCFDEAKQGLCGR-

Paracoccus denitrificans Gdh
MGSSHHHHHHSSGLVPRGSHMPQIDDKLAPIYEEVVRRNAGEPEFHQAVREVLESLGRVVAKRPDYLEDALIERICEPERQIIFRVPWTDDKGRVQI
NRGFRVQFSSAMGPYKGGLRFHPSVNVGIIKFLGFEQIFKNALTGLPIGGGKGGSDFDPKGRSDGEIMRFCQSFMTELYRHLGEYTDVPAGDIGVGA
REIGYMFGQYKRLTNRYEAGVLTGKGLFYGGSLARKEATGYGNTYFTQAMLKTGGTDFDGKTVVVSGSGNVAIYTIEKVQEFGGKVIACSDSSGYIV
DEAGIDLALVKEIKEVRRGRISQYVRMKGEGNGAYFVKSGEGSIWDVACEVAMPSATQNELTGKDAAKLVKNGVTAVGEGANMPCTPEAIRAFQQAG
VKFGPGKAANAGGVATSALEMQQNASRDRWSFEKTEAKLAEIMRDIHDSCYSTAEEFGAPGDYVIGANIAGFIRVAEPMRAFGVI-

Stigmatella aurantiaca βCal
MGSSHHHHHHSSGLVPRGSHMSTKAIIRLRMSSHDAHYGGNLVDGARMLGLFGDVATELCIRHDGDEGLFRAYDSVEFLAPVYAGDFIEAEGEILSE
GNTSRKMRFEARKVIRPRTDVNDSAADLLSEPVVVCRATGTCVVPKDKQRIPR-

Erythrobacter NAP1 Pcs
MGHHHHHHHHHHSSGHIEGRHMIGEGDDIGSSNNLEKQSHGLRISDRDHFQRLREECRSDPGEFHGRLAKREICWLIEGPGGNPAWAFYDDAAETWT
GWDASSAAPITLDLPESFEPWERAFNDDDPPNWRWFEGGLTSTAFNEVDRHVLSGHGDEAAMIFEGDRWNMASEGGRGGPVDSEVISRRKLLLESAK
CALALKALGLEAGDRIALNMPSIPEQIYWTEGAKRMGIVYTPVFGGFSDKTLSDRIADAGARVVVTADGSYRNAQMVPFKPSYTDPALDNFIAVPVA
MELLGQALEDGELVVAPEHAGLIRSEVAGLLDGEVTVERSDVMRGVGKALTAIASGEAAGGAMTPRQAAQLRIAIASALVDSPPRVDAVVVVKHTAQ
PDLPWNEARDHWSHDLTAAAGEELLKAARDAGFDVADEEALLALSDTEFVRAIWAGAPVLAVDAEYPNFIIYTSGSTGKPKGVVHVHGGYASGVAAT
MPAAFGAEPGDVMYVVADPGWITGQSYQIAASLLSRVTTVITEGSPVFPHAGRFASIIERYGVNVFKAGVTFLKSVMQNPENLKDIQRYDLSSLKVA
TFCAEPVSPAVQAFAMEHITHRYINSYWATEHGGMVWTHFADADGFPLEADAHTYPLPWIMGDVWVEDADGSSNGPVEYERDTGTGGAPWRVAEDGE
KGEIVIALPYPYLTRTIWGDVENFTVEHVGNLARVAGGWRGDEVRYADTYWRRWKGAWAYTQGDFAMRHPDGSFSLHGRSDDVINVSGHRIGTEEIE
GAILRDKALDPNSPVGNVIVIGAPHSQKGVTPIAFVTPVEGRRLTQDDKRRLTDLVRTEKGAVAVPQDFIELSEFPETRSGKYMRRMVRAVVEGGEV
GDASTLRNPESLDELARAVDGWKRRQSLSDTQALFERYRFFTIQYNLVAPGKRVATVTVKNPPVNALNERALDELVIIAEHLARKDDVAAVVFTGSG
TASFVAGADIRQMLEEVNSVEEAKALPDNAQLAFRTIEEMDKPCIAAIQGVALGGGMEFALACHYRVAEPKARFGQPEINLRLLPGYGGTQRLPRLL
ADGGGETGLRDALDLILGGRAIDADAALAVGAVDALADGSDNALSHAHAMVREFVRSGDDSALGKAFAARKTQTQSWHEPASIDLDAVLEDEFLQRI
LNQLEWAGRDKAGERALDAVRTGWTQGMTAGLECEAQRFAEAIIDPEGGKTGIQQFMDKQSPPLPVRRDGVWEDDQHEATKTALIEAGDLLPLGAPF
YPGVTAIPPKQLAFGIARDPDTGAPRFGPPETHERELVVNTPKPGANEALIYLLSSEVNFNDIWALTGIPVSPFDAHDEDVQITGSGGLALVAALGS
ELKEEGRLQVGDLVSVYSGTSELLSPLAGDDPMYAGFAIQGYETKTGSHAQFLTVQGPQLHRPPADLTLEQAGAYTLNLGTVARCLFTTLEIQAGKT
AFVEGSATGTGLDALKSSVRTGLAVTGLVSSEDRAEFVKSHGSVGAINRKDPEIADCFTPVPDDPDEARQWEADGEKLLDAYRETNGGKLADYVVSH
AGERAFPRSFQLLAEGGRLAFYGASSGYHFSFMGKGGEARPDEMLARANLRGGESVLLYYGPGSHELADEKGLEMVEAARLMKARMVIVTTSDGQRE
FLQSLGLEDAVEGIVSIEGLKRRLSDFHWPDTLPRLPDARTDIENFKIGVRAYQQNTMKPFGTAVGKLLRSPGNPRGVPDLVIERAGQDTLGVSTSL
VKPFGGRVIYAEEMAGRRYTFYAPQVWTRQRRIYMPSAEIFGTALCNAYEVTMMNEMVAAGLLDVTEPTMVPWEGLPEAHQAMWDNRHSGATYVVNH
ALPAMGLTTKDELLEYWVAAQSDTGETS-

Methylorubrum extorquens Pcc
MGSSHHHHHHSSGLVPRGSHMKDILEKLEERRAQARLGGGEKRLEAQHKRGKLTARERIELLLDHGSFEEFDMFVQHRSTDFGMEKQKIPGDGVVTG
WGTVNGRTVFLFSKDFTVFGGSLSEAHAAKIVKVQDMALKMRAPIIGIFDAGGARIQEGVAALGGYGEVFRRNVAASGVIPQISVIMGPCAGGDVYS
PAMTDFIFMVRDTSYMFVTGPDVVKTVTNEVVTAEELGGAKVHTSKSSIADGSFENDVEAILQIRRLLDFLPANNIEGVPEIESFDDVNRLDKSLDT
LIPDNPNKPYDMGELIRRVVDEGDFFEIQAAYARNIITGFGRVEGRTVGFVANQPLVLAGVLDSDASRKAARFVRFCNAFSIPIVTFVDVPGFLPGT
AQEYGGLIKHGAKLLFAYSQATVPLVTIITRKAFGGAYDVMASKHVGADLNYAWPTAQIAVMGAKGAVEIIFRAEIGDADKIAERTKEYEDRFLSPF
VAAERGYIDEVIMPHSTRKRIARALGMLRTKEMEQPWKKHDNIPL-

MFDKILIANRGEIACRIIKTAQKMGIKTVAVYSDADRDAVHVAMADEAVHIGPAPAAQSYLLIEKIIDACKQTGAQAVHPGYGFLSERESFPKALAE
AGIVFIGPNPGAIAAMGDKIESKKAAAAAEVSTVPGFLGVIESPEHAVTIADEIGYPVMIKASAGGGGKGMRIAESADEVAEGFARAKSEASSSFGD
DRVFVEKFITDPRHIEIQVIGDKHGNVIYLGERECSIQRRNQKVIEEAPSPLLDEETRRKMGEQAVALAKAVNYDSAGTVEFVAGQDKSFYFLEMNT
RLQVEHPVTEMITGLDLVELMIRVAAGEKLPLSQDQVKLDGWAVESRVYAEDPTRNFLPSIGRLTTYQPPEEGPLGGAIVRNDTGVEEGGEIAIHYD
PMIAKLVTWAPTRLEAIEAQATALDAFAIEGIRHNIPFLATLMAHPRWRDGRLSTGFIKEEFPEGFIAPEPEGPVAHRLAAVAAAIDHKLNIRKRGI
SGQMRDPSLLTFQRERVVVLSGQRFNVTVDPDGDDLLVTFDDGTTAPVRSAWRPGAPVWSGTVGDQSVAIQVRPLLNGVFLQHAGAAAEARVFTRRE
AELADLMPVKENAGSGKQLLCPMPGLVKQIMVSEGQEVKNGEPLAIVEAMKMENVLRAERDGTISKIAAKEGDSLAVDAVILEFA-

Staphylococcus schweitzeri Dsr
MGSSHHHHHHSSGLVPRGSHMPKIVVVGAVAGGATCASQIRRLDKESDIIIFEKDRDMSFANCALPYVIGEVVEDRRLALAYTPEKFYNRKQITVKT
YHEVIAINDEKQTVSVLNRKTNEQFEESYDKLILSPGASANSLGFESDITFTLRNLEDTDAIDQFIKANQVDKVLVVGAGYVSLEVLENLYERGLHP
TLIHRSDKINKLMDADMNQPILDELDKREIPYRLNEEIEAINGNEITFKSGKVEHYDMIIEGVGTHPNSKFIESSNIKLDRKGFIPVNDKFETNVPN

IYAIGDIATSHYRHVDLPASVPLAWGAHRAASIVAEQIAGNDTIEFKGFLGNNIVKFFDYTFASVGVKPNELKQFDYKMVEVTQGAHANYYPGNSPL
HLRVYYDTTSRQILRAASVGKEGADKRIDVLSMAMMNQLTVDELTEFEVAYAPPYSHPKDLINMIGYKAK-

Escherichia coli KatE
MRGSHHHHHHTDPALRASQHNEKNPHQHQSPLHDSSEAKPGMDSLAPEDGSHRPAAEPTPPGAQPTAPGSLKAPDTRNEKLNSLEDVRKGSENYALT
TNQGVRIADDQNSLRAGSRGPTLLEDFILREKITHFDHERIPERIVHARGSAAHGYFQPYKSLSDITKADFLSDPNKITPVFVRFSTVQGGAGSADT
VRDIRGFATKFYTEEGIFDLVGNNTPIFFIQDAHKFPDFVHAVKPEPHWAIPQGQSAHDTFWDYVSLQPETLHNVMWAMSDRGIPRSYRTMEGFGIH
TFRLINAEGKATFVRFHWKPLAGKASLVWDEAQKLTGRDPDFHRRELWEAIEAGDFPEYELGFQLIPEEDEFKFDFDLLDPTKLIPEELVPVQRVGK
MVLNRNPDNFFAENEQAAFHPGHIVPGLDFTNDPLLQGRLFSYTDTQISRLGGPNFHEIPINRPTCPYHNFQRDGMHRMGIDTNPANYEPNSINDNW
PRETPPGPKRGGFESYQERVEGNKVRERSPSFGEYYSHPRLFWLSQTPFEQRHIVDGFSFELSKVVRPYIRERVVDQLAHIDLTLAQAVAKNLGIEL
TDDQLNITPPPDVNGLKKDPSLSLYAIPDGDVKGRVVAILLNDEVRSADLLAILKALKAKGVHAKLLYSRMGEVTADDGTVLPIAATFAGAPSLTVD
AVIVPCGNIADIADNGDANYYLMEAYKHLKPIALAGDARKFKATIKIADQGEEGIVEADSADGSFMDELLTLMAAHRVWSRIPKIDKIPAGLCGR-

Mycolicibacterium vaccae Fdh(D221A)
MAKVLCVLYDDPVDGYPKTYARDDLPKIDHYPGGQILPTPKAIDFTPGQLLGSVSGELGLREYLESNGHTLVVTSDKDGPDSVFERELVDADVVISQ
PFWPAYLTPERIAKAKNLKLALTAGIGSDHVDLQSAIDRNVTVAEVTYCNSISVAEHVVMMILSLVRNYLPSHEWARKGGWNIADCVSHAYDLEAMH
VGTVAAGRIGLAVLRRLAPFDVHLHYTARHRLPESVEKELNLTWHATREDMYPVCDVVTLNCPLHPETEHMINDETLKLFKRGAYIVNTARGKLCDR
DAVARALESGRLAGYAGDVWFPQPAPKDHPWRTMPYNGMTPHISGTTLTAQARYAAGTREILECFFEGRPIRDEYLIVQGGALAGTGAHSYSKGNAT
GGSEEAAKFKKAAENSSSVYRGAAALEHHHHHH-

Escherichia coli AhpFC
MGSSHHHHHHSQDPLDTNMKTQLKAYLEKLTKPVELIATLDDSAKSAEIKELLAEIAELSDKVTFKEDNSLPVRKPSFLITNPGSNQGPRFAGSPLG
HEFTSLVLALLWTGGHPSKEAQSLLEQIRHIDGDFEFETYYSLSCHNCPDVVQALNLMSVLNPRIKHTAIDGGTFQNEITDRNVMGVPAVFVNGKEF
GQGRMTLTEIVAKIDTGAEKRAAEELNKRDAYDVLIVGSGPAGAAAAIYSARKGIRTGLMGERFGGQILDTVDIENYISVPKTEGQKLAGALKVHVD
EYDVDVIDSQSASKLIPAAVEGGLHQIETASGAVLKARSIIVATGAKWRNMNVPGEDQYRTKGVTYCPHCDGPLFKGKRVAVIGGGNSGVEAAIDLA
GIVEHVTLLEFAPEMKADQVLQDKLRSLKNVDIILNAQTTEVKGDGSKVVGLEYRDRVSGDIHNIELAGIFVQIGLLPNTNWLEGAVERNRMGEIII
DAKCETNVKGVFAAGDCTTVPYKQIIIATGEGAKASLSAFDYLIRTKTA-

MSLINTKIKPFKNQAFKNGEFIEITEKDTEGRWSVFFFYPADFTFVCPTELGDVADHYEELQKLGVDVYAVSTDTHFTHKAWHSSSETIAKIKYAMI
GDPTGALTRNFDNMREDEGLADRATFVVDPQGIIQAIEVTAEGIGRDASDLLRKIKAAQYVASHPGEVCPAKWKEGEATLAPSLDLVGKI-

Escherichia coli YjeF
MRGSHHHHHHTDPALRATDHTMKKNPVSIPHTVWYADDIRRGEREAADVLGLTLYELMLRAGEAAFQVCRSAYPDARHWLVLCGHGNNGGDGYVVAR
LAKAVGIEVTLLAQESDKPLPEEAALAREAWLNAGGEIHASNIVWPESVDLIVDALLGTGLRQAPRESISQLIDHANSHPAPIVAVDIPSGLLAETG
ATPGAVINADHTITFIALKPGLLTGKARDVTGQLHFDSLGLDSWLAGQETKIQRFSAEQLSHWLKPRRPTSHKGDHGRLVIIGGDHGTAGAIRMTGE
AALRAGAGLVRVLTRSENIAPLLTARPELMVHELTMDSLTESLEWADVVVIGPGLGQQEWGKKALQKVENFRKPMLWDADALNLLAINPDKRHNRVI
TPHPGEAARLLGCSVAEIESDRLHCAKRLVQRYGGVAVLKGAGTVVAAHPDALGIIDAGNAGMASGGMGDVLSGIIGALLGQKLSPYDAACAGCVAH
GAAADVLAARFGTRGMLATDLFSTLQRIVNPEVTDKNHDESSNSAPGLCGR-

Escherichia coli CoaE
MRGSHHHHHHTDPALRARYIVALTGGIGSGKSTVANAFADLGINVIDADIIARQVVEPGAPALHAIADHFGANMIAADGTLQRRALRERIFANPEEK
NWLNALLHPLIQQETQHQIQQATSPYVLWVVPLLVENSLYKKANRVLVVDVSPETQLKRTMQRDDVTREHVEQILAAQATREARLAVADDVIDNNGA
PDAIASDVARLHAHYLQLASQFVSQEKPGLCGR-

Cereibacter sphaeroides CsMcd
MGSSHHHHHHSSGLVPRGSHMTGQPLLGDLLTLASDALPEVEALFETARSALKERVTTDGKVSSKALEEEQFAAHALSWLATYVESLRQMRAWAGRL
ETEGRFGEMEALILQIAFGEYLAQIRGGIPMSQTETARVQDIGIELGHPGEAVRRLIQAGNTPAARARLVALMRDNHGRATFGASGLDEELEMIRDQ
FRRFADERVAPHAHGWHMRDELIPMEIVEALAEMGVFGLTIPEEFGGFGLSKASMVVVSEELSRGYIGVGSLGTRSEIAAELILCGGTDAQKAAWLP
KLASGEILPTAVFTEPNTGSDLGSLRTRAVKDGDEWVVHGNKTWITHAARTHVMTLLARTDLETTDYRGLSMFLAEKVPGTDADPFPTPGMTGGEIE
VLGYRGMKEYEIGFDGFRVKAENLLGGVEGQGFKQLMQTFESARIQTAARAIGVAQNALEVGMQYAEERKQFGKALIEFPRVAGKLAMMAVEIMVAR
QLTYHSAWEKDHGQRCDLEAGMAKLLGARVAWAAADNALQIHGGNGFALEYQISRILCDARILNIFEGAAEIQAQVIARRLLD-

Paracoccus denitrificans CsMcd
MGSSHHHHHHSSGLVPRGSHMKDMPAMPADTPSALLALAGEALPELESLQSRATEALRALVAPAGKPQPALLEQHQHAAHALSWLTTYVESIRQLSG
WAGRLAEAGNLGRIEALILQIGLGEYLGQIAGGIPMSQTEFARLSDLELDWQPGEAAAKLMRGNTAPARAELARLMQDNHGRATFGATGLDEDLEMI
RDQFRRYAEERVIPNAHEWHLKDQLIPMEIIEELAELGVFGLTIPEEFGGLGLSKASMVVVTEELSRGYIGVGSLGTRSEIAAELILCGGTEAQKAK
WLPGLASGEILSTAVFTEPNTGSDLGSLRTRAVRDGEDWVVTGNKTWITHAQRTHVMTLLARTDPETTDWRGLSMFLAEKEPGTDDDPFPTPGMTGG
EIEVLGYRGMKEYELGFDGFRIKGENLLGGEPGRGFKQLMETFESARIQTAARAVGVAQSAAEIGMRYAVDRKQFGKSLIEFPRVADKLAMMAVEIM
IARQLTYFSAWEKDHGRRCDLEAGMAKLLGARVAWAAADNALQIHGGNGFALEYAISRVLCDARILNIFEGAAEIQAQVIARRLLD-

Streptomyces albus SaMcd
MGSSHHHHHHSSGLVPRGSHMSRLAQTHGLTDIQQEILSTVRDFVDKEIIPVATELEHRDEYPTQIVEGLKELGLFGLMIPEEYGGLGESLLTYALC
VEEIARGWMSVSGIINTHFIVAYMLKQHGTQEQREYFLPRMATGEVRGAFSMSEPALGSDVSAISTKGVKVGDEYALTGQKMWLTNGGSSTLVAVLC
RTDEGHPEGTAPHKSMTTFLVEKEPGFGEVRPGLTIPGKIEKMGYKGVDTTEMILDGLRIPANRVLGGTTGRGFYQMMDGVEVGRVNVAARGCGVAQ
RAFELGVSYAQQRHTFGKPIAQHQAIQFKLAEMATKVEAAHAMMVNAARKKDSGERNDLEAGMAKYLASEYCKEVVEDAFRIHGGYGFSKEYEIERL
YREAPMLLIGEGTAEIQKMIIGRRLLEEYRFQG-

Leptospira interrogans LiMcd
MGSSHHHHHHSSGLVPRGSHMSAIKTIDQTTAKKALTVSAGVIEEVTKALAARCSVNGKVSVDKMDENQLVQYQIAWLTSEQRIAEKFIEYAWDSSR
GTGDLEQEMAVVFAAETVNHIRSEISSRPSEYGIKSSDLVSKIFNDEINQFLENAMAIQNYNEIAEKIVAKGHFGAYGLDEDHEMFRETFKKFAEDV
VIPHAEHVHRHDDIIPEDIIGGLKEMGCFGLCIPESYGGIQPNDKPDNLSMLVVTEELSRGGLGIAGSLITRPEIMSKALLKGGTQEQKDKWLPLLA
SGERMAGIMVTEPNYGSDVAGVSVTAKPANGGWVINGVKTWCTFAGYANLLLILCRTESDPSLKHKGLSILLAEKPTFTGHEFTYTQPEGGKIEGKA
IGTIGYRGMHSFEVSFDNYFVPAENLLGGEAGRGKGFYFQMEGFAGGRIQTAARAHGVMQAALEAALRYARERAVFQKPIYEYNLTKYKIARMAVIL
QASRQYANHVANLLDNHKGQMEATLIKFYASKVAEWVTREAMQIHGGMGYAEEYAVSRYFVDARVFSIFEGAEEVMALRVIAKSLMDQYSAS-

Wenxinia marina WmMcd
MGSSHHHHHHSSGLVPRGSHMAHDGQDASTDAVLGGLTDLTRAALAPVADVLEEATAAVKRRIGNGPLDAQQDAAHGLSWLATYGRALQQMQGWADR
LEEQGRLGEAEQLVLQIAFGEYLAQIQGGIPMSQGEIVRLEALGADPSPLDADPVRRLIAEGNSDAARLRLAALLAERGGDCAASGLDDEMEMIRDQ
FRRFAAEKVEPFAHDWHLKDELIPMALIEELSEMGVFGLTIPEEYGGLGLPKTAMAVVSEELSRGYIGVGSLGTRSEIAAELILGGGTEAQKAHWLP
KIASGAVLPTAVFTEPNTGSDLGALRTRAVPDGEGWRITGNKTWITHAARAGLMTLLARTDPETDDYRGLSMFLAEKTPGTDAEPFPDAGLTGGEIG
VLGYRGMKEYELAFDGFRVSGDGLLGGAPGTGFKQLMQTFESARIQTAARAVGVAQAALDVALSYAQERRQFGRPLIAFPRVAGKLAMMAAEIMVAR
QLTYHSAREKDAGHRCDLEAGMAKLLAARVAWAAADNGLQIHGGNGFALEYRISRLLCDARILNIFEGAAEIQAQVIARRLLG

Frankia sp. FsMcd
MGSSHHHHHHSSGLVPRGSHMGRIAQTDGLTDVQTDILAAVRTFVDKEILPHANELERKDEFPDAIVEAMKEMGLFGITIPEQYGGLGESLLTYALV
VEEIARGWMSVSGVINTHFIVAYLVLQHGTEEQRQRLLPKMATGEVRGAFSMSEPGCGSDVSAITTRADRDGDDYVINGQKMWLTNGARAGVVATLV
KTDEGADSVYRNMTTFLLEKEPGFGTHGGITIPGKLDKLGYKGVETTEMILDGHRTPASSILGGPEAAGRGFYQMMDGVEVGRVNVAARACGIMIRA
FELAIAYAQQRRTFGHQIADHQAIAFKLADMATKVEAGHLMMVSAARKKDSGQRNDVEAGMAKYLASEYCHEVTTESFRIHGGYGYSKEYEIERLYR
EAPFMLIGEGTSEVQKRIISRALLKEYKLPG-

Erythrobacter litoralis ElMcd
MGSSHHHHHHSSGLVPRGSHMSDWIECANDAAAAARDFAETVRLRVHERVAPGGHVDADLVTLEQHAVHGFAWIAATTAALEATVDWAKRARSQGHF
GRVEELTLRIGFGEYCVQLVSGVPMSAGEIVRQQALGVSVEAAAMASDPAVARFLKDGNTPETRAEFAALLAEGARPDEGLGDETLDLVRAQFRAFT
ADRIAPHAHGWHLADALIPAEVIAEMAQLGVFGVCIDEKYGGLGLGKLAMSVVSEELSRGWICAGSLGTRSEIAGELIGENGTEAQKAHWLPRIADG
SVLPTAVFTEPDTGSDLASVRTRARRQADGTWRVDGAKTWITHAARADLMTLIARTDPDAPGYKGLSMFLAAKTRGSDADPFPDPGIDGSEIEVLGY
RGMKEYALGFDGFAVAGDGLLGGAEGQGFKQLMRTFEGARIQTAARAVGVAWNAFDLALDYAMGRRQFSEPLTAFPRVADKLAMMATETVMSRELTY
YAARAKDRGARCDIEAGMAKLLAARTAWSAADNAVQIHGGNGYALEYPISRVLCDARILNIFEGAAEIQAQVIARGLLAAQAPPREAEPVRQSA-

Caulobacter vibrioides CvMcd
MGSSHHHHHHSSGLVPRGSHMTTTIARDDAENLVLPGLTGLLREAADATALFVAEAKPAVLAHIAPEGGKVDRKLADVHQHRVHGYGWYAAYAELLN
QVAGWAERLEAEGRFGEIEALLAQLLFSEYCAQLVGGVPMNQGEIIRPAHLVEDPAILARLSSSAAATLIAEGGTQAVKSRVAQRLAEARGRPTLEH
TGLDETFEMIRDQFHAFAEEKVTPFAHEWHLKDELIPIELVEELGALGVFGLTIPEEYGGSGMGKTAMCVVSEELSRAWIGVGSLATRSEIAGELIL
TGGTEEQKQYWLPKIASAEILPTAVFTEPNTGSDLGALRTRAELKGDHYVVTGNKTWITHAARADVMTLLVRTDPATTDYRGLSMLLAPKPRGTDEA
PFPAEGMSGGEIGVIGYRGMKEYELGFDGFTVPAENLLGGAPGQGFKQLMATFESARIQTAARAVGVAQAALEVGLGYALDRKQFGQAIFAFPRVAN
KLAMMAAEIMGVRQLTYFAARQKDEGKRCDLEAGMAKLIAARVAWAAADNALQIHGGNGFAMEYAASRLLADARILNIFEGAGEIQAQVIARRLLDG
GN-

Bacterium HR19 HrMcd
MGSSHHHHHHSSGLVPRGSHMEVIRDGKFYEEIRRQVREFAESEVKPIAHKYDREDKDIPWDVLKKMAELGYFGILVPEEWGGLGLDYMSMAIVAEE
LSRVWLSVGSVMTRNLIAETLLLNNGLEEQKKKYLPSLARGEIFAAAAFTEPNAGSDTAGMKLKAEKVKGGWILNGTKTWCTFANRANILVVLARTD
PNPPKRHLGLSIFIVEKEPSEAHEKIKHPNIHGELIETVGYHGMHCWTLHFEDCFVPDENLLGGEPGKGFYQLMATYESARIQTAARAIGVAQGAFE
LAVKYAKERYQFGKPIADFQLIRSKLAKMMTYIEAARQLTYYACRMKDTGKRCDLEAGMAKLFAAEMVEYVTSEAMQIFGGYGYSKEYEIERYWRDG
RLFKIFEGTSEIQEEVIAKRLLEIY-

Cupriavidus necator Act
MGSSHHHHHHSSGLVPRGSHMTDVVIVSAARTAVGKFGGSLAKIPAPELGAVVIKAALERAGVKPEQVSEVIMGQVLTAGSGQNPARQAAIKAGLPA
MVPAMTINKVCGSGLKAVMLAANAIMAGDAEIVVAGGQENMSAAPHVLPGSRDGFRMGDAKLVDTMIVDGLWDVYNQYHMGITAENVAKEYGITREA
QDEFAVGSQNKAEAAQKAGKFDEEIVPVLIPQRKGDPVAFKTDEFVRQGATLDSMSGLKPAFDKAGTVTAANASGLNDGAAAVVVMSAAKAKELGLT
PLATIKSYANAGVDPKVMGMGPVPASKRALSRAEWTPQDLDLMEINEAFAAQALAVHQQMGWDTSKVNVNGGAIAIGHPIGASGCRILVTLLHEMKR
RDAKKGLASLCIGGGMGVALAVERK-

Cupriavidus necator Pct
MGSSHHHHHHSSGLVPRGSHMKVITAREAAALVQDGWTVASAGFVGAGHAEAVTEALEQRFLQSGLPRDLTLVYSAGQGDRGARGVNHFGNAGMTAS
IVGGHWRSATRLATLAMAEQCEGYNLPQGVLTHLYRAIAGGKPGVMTKIGLHTFVDPRTAQDARYHGGAVNERARQAIAGGKACWVDAVDFRGEEYL
FYPSFPIHCALIRCTAADTRGNLSTHREAFHHELLAMAQAAHNSGGIVIAQVESLVDHHEILQAIHVPGILVDYVVVCDNPANHQMTFAESYNPAYV
TPWQGEAAVVEAEATPVAAGPLDARTIVQRRAVMELARRAPRVVNLGVGMPAAVGMLAHQAGLDGFTLTVEAGPIGGTPADGLSFGASAYPEAVVDQ
PAQFDFYEGGGIDLAILGLAELDGHGNVNVSKFGEGEGASIAGVGGFINITQSARAVVFMGTLTAGGLEVRAGEGRLQIVREGRVKKIVPEVSHLSF
NGPYVASLGIPVLYITERAVFEMRAGAGGEARLTLVEIAPGVDLQRDVLDQCATPVAVAPDLREMDARLFQAGPLHL-

Anaerotignum propionicum βct
MGHHHHHHHHHHSSGHIEGRHMLEMRKVPIITADEAAKLIKDGDTVTTSGFVGNAIPEALDRAVEKRFLETGEPKNITYVYCGSQGNRDGRGAEHFA
HEGLLKRYIAGHWATVPALGKMAMENKMEAYNVSQGALCHLFRDIASHKPGVFTKVGIGTFIDPRNGGGKVNDITKEDIVELVEIKGQEYLFYPAFP

IHVALIRGTYADESGNITFEKEVAPLEGTSVCQAVKNSGGIVVVQVERVVKAGTLDPRHVKVPGIYVDYVVVADPEDHQQSLDCEYDPALSGEHRRP
EVVGEPLPLSAKKVIGRRGAIELEKDVAVNLGVGAPEYVASVADEEGIVDFMTLTAESGAIGGVPAGGVRFGASYNADALIDQGYQFDYYDGGGLDL
CYLGLAECDEKGNINVSRFGPRIAGCGGFINITQNTPKVFFCGTFTAGGLKVKIEDGKVIIVQEGKQKKFLKAVEQITFNGDVALANKQQVTYITER
CVFLLKEDGLHLSEIAPGIDLQTQILDVMDFAPIIDRDANGQIKLMDAALFAEGLMGLKEMKS-

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