Complete-MFA: Complementary parallel labeling experiments technique for metabolic flux analysis

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Figure S7. Inconsistent metabolic fluxes were obtained when fluxes were estimated using an incomplete metabolic network model without the CO_2 exchange reaction (v_{71}).

Figure S8. Comparison of the precision of metabolic fluxes estimated using combined analysis of dual ^{13}C-tracer experiments.

Table S1. Metabolic network model of *E. coli* used for ^{13}C metabolic flux analysis

Glycolysis

v_1 Gluc.ext (abcdef) + PEP (ghi) → G6P (abcdef) + Pyr (ghi)

v_2 G6P (abcdef) ↔ F6P (abcdef)

v_3 F6P (abcdef) + ATP → FBP (abcdef)

v_4 FBP (abcdef) ↔ DHAP (cba) + GAP (def)

v_5 DHAP (abc) ↔ GAP (abc)

v_6 GAP (abc) ↔ 3PG (abc) + ATP + NADH

v_7 3PG (abc) ↔ PEP (abc)

v_8 PEP (abc) → Pyr (abc) + ATP

Pentose Phosphate Pathway

v_9 G6P (abcdef) → 6PG (abcdef) + NADPH

v_{10} 6PG (abcdef) → Ru5P (bcdef) + CO2 (a) + NADPH

v_{11} Ru5P (abcde) ↔ X5P (abcde)

v_{12} Ru5P (abcde) ↔ R5P (abcde)

v_{13} X5P (abcde) ↔ TK-C2 (ab) + GAP (cde)

v_{14} F6P (abcdef) ↔ TK-C2 (ab) + E4P (cdef)

v_{15} S7P (abcdefg) ↔ TK-C2 (ab) + R5P (cdefg)

v_{16} F6P (abcdef) ↔ TA-C3 (abc) + GAP (def)

v_{17} S7P (abcdefg) ↔ TA-C3 (abc) + E4P (defg)

Entner-Doudoroff Pathway

v_{18} 6PG (abcdef) → KDPG (abcdef)

v_{19} KDPG (abcdef) → Pyr (abc) + GAP (def)

TCA Cycle

v_{20} Pyr (abc) → AcCoA (bc) + CO2 (a) + NADH

v_{21} OAC (abcd) + AcCoA (ef) → Cit (dcbfea)

v_{22} Cit (abcdef) ↔ ICit (abcdef)

v_{23} ICit (abcdef) ↔ AKG (abcde) + CO2 (f) + NADPH

v_{24} AKG (abcde) → SucCoA (bcde) + CO2 (a) + NADH

v_{25} SucCoA (abcd) ↔ Suc (½ abcd + ½ dcba) + ATP

v_{26} Suc (½ abcd + ½ dcba) ↔ Fum (½ abcd + ½ dcba) + FADH2

v_{27} Fum (½ abcd + ½ dcba) ↔ Mal (abcd)

v_{28} Mal (abcd) ↔ OAC (abcd) + NADH

Glyoxylate Shunt

v_{29} ICit (abcdef) → Glyox (ab) + Suc (½ edcf + ½ fcde)

v_{30} Glyox (ab) + AcCoA (cd) → Mal (abdc)

Amphibolic Reactions

v_{31} Mal (abcd) → Pyr (abc) + CO2 (d) + NADPH

v_{32} Mal (abcd) → Pyr (abc) + CO2 (d) + NADH

v_{33} PEP (abc) + CO2 (d) → OAC (abcd)

v_{34} OAC (abcd) + ATP → PEP (abc) + CO2 (d)

Acetic Acid Formation

v_{35} AcCoA (ab) ↔ Ac (ab) + ATP

Amino Acid Biosynthesis

v_{36} AKG (abcde) + NADPH + NH3 → Glu (abcde)

v_{37} Glu (abcde) + ATP + NH3 → Gln (abcde)

v_{38} Glu (abcde) + ATP + 2 NADPH → Pro (abcde)

v_{39} Glu (abcde) + CO2 (f) + Gln (ghijk) + Asp (lmno) + AcCoA (pq) + 5 ATP + NADPH →

Arg (abcdef) + AKG (ghijk) + Fum (lmno) + Ac (pq)

v_{40} OAC (abcd) + Glu (efghi) → Asp (abcd) + AKG (efghi)

v_{41} Asp (abcd) + 2 ATP + NH3 → Asn (abcd)

v_{42} Pyr (abc) + Glu (defgh) → Ala (abc) + AKG (defgh)

v_{43} 3PG (abc) + Glu (defgh) → Ser (abc) + AKG (defgh) + NADH

v_{44} Ser (abc) ↔ Gly (ab) + MEETHF (c)

v_{45} Gly (ab) ↔ CO2 (a) + MEETHF (b) + NADH + NH3

v_{46} Thr (abcd) → Gly (ab) + AcCoA (cd) + NADH

v_{47} Ser (abc) + AcCoA (de) + 3 ATP + 4 NADPH + SO4 → Cys (abc) + Ac (de)

v_{48} Asp (abcd) + Pyr (efg) + Glu (hijkl) + SucCoA (mnop) + ATP + 2 NADPH →

LL-DAP (½ abcdgfe + ½ efgdcba) + AKG (hijkl) + Suc (½ mnop + ½ ponm)

v_{49} LL-DAP (½ abcdefg + ½ gfedcba) → Lys (abcdef) + CO2 (g)

v_{50} Asp (abcd) + 2 ATP + 2 NADPH → Thr (abcd)

v_{51} Asp (abcd) + METHF (e) + Cys (fgh) + SucCoA (ijkl) + ATP + 2 NADPH →

Met (abcde) + Pyr (fgh) + Suc (½ ijkl + ½ lkji) + NH3

v_{52} Pyr (abc) + Pyr (def) + Glu (ghijk) + NADPH → Val (abcef) + CO2 (d) + AKG (ghijk)

v_{53} AcCoA (ab) + Pyr (cde) + Pyr (fgh) + Glu (ijklm) + NADPH →

Leu (abdghe) + CO2 (c) + CO2 (f) + AKG (ijklm) + NADH

v_{54} Thr (abcd) + Pyr (efg) + Glu (hijkl) + NADPH → Ile (abfcdg) + CO2 (e) + AKG (hijkl) + NH3

v_{55} PEP (abc) + PEP (def) + E4P (ghij) + Glu (klmno) + ATP + NADPH →

Phe (abcefghij) + CO2 (d) + AKG (klmno)

v_{56} PEP (abc) + PEP (def) + E4P (ghij) + Glu (klmno) + ATP + NADPH →

Tyr (abcefghij) + CO2 (d) + AKG (klmno) + NADH

v_{57} Ser (abc) + R5P (defgh) + PEP (ijk) + E4P (lmno) + PEP (pqr) + Gln (stuvw) + 3 ATP + NADPH →

Trp (abcedklmnoj) + CO2 (i) + GAP (fgh) + Pyr (pqr) + Glu (stuvw)

v_{58} R5P (abcde) + FTHF (f) + Gln (ghijk) + Asp (lmno) + 5 ATP →

His (edcbaf) + AKG (ghijk) + Fum (lmno) + 2 NADH

One-Carbon Metabolism

v_{59} MEETHF (a) + NADH → METHF (a)

v_{60} MEETHF (a) → FTHF (a) + NADPH

Oxidative Phosphorylation

v_{61} NADH + ½ O2 → 2 ATP

v_{62} FADH2 + ½ O2 → 1 ATP

Transhydrogenation

v_{63} NADH ↔ NADPH

ATP Hydrolysis

v_{64} ATP → ATP:ext

Transport

v_{65} Ac (ab) → Ac.ext (ab)

v_{66} CO2 (a) → CO2.ext (a)

v_{67} O2.ext → O2

v_{68} NH3.ext → NH3

v_{69} SO4.ext → SO4

Biomass Formation

v_{70} 0.488 Ala + 0.281 Arg + 0.229 Asn + 0.229 Asp + 0.087 Cys + 0.250 Glu + 0.250 Gln + 0.582 Gly + 0.090 His + 0.276 Ile + 0.428 Leu + 0.326 Lys + 0.146 Met + 0.176 Phe + 0.210 Pro + 0.205 Ser + 0.241 Thr + 0.054 Trp + 0.131 Tyr + 0.402 Val + 0.205 G6P + 0.071 F6P + 0.754 R5P + 0.129 GAP + 0.619 3PG + 0.051 PEP + 0.083 Pyr + 2.510 AcCoA + 0.087 AKG + 0.340 OAC + 0.443 MEETHF + 33.247 ATP + 5.363 NADPH $\rightarrow$ 39.68 Biomass + 1.455 NADH

CO_2 Exchange

v_{71} CO_2.unlabeled (a) + CO_2 (b) $\rightarrow$ CO_2 (a) + CO_2.out (b)

The net effect of reaction v_{71} is exchange of intracellular CO_2 for an unlabeled CO_2 without affecting intracellular carbon balances.

Table S2. Biomass measurements, glucose measurements, and estimated growth rates.

BIOMASS MEASUREMENTS (OD600)

Time (hr)	[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc
2.0	n/a	n/a	n/a	n/a	n/a	n/a
8.0	0.161	0.175	0.175	0.154	0.179	0.208
10.1	0.756	0.777	0.809	0.686	0.704	0.813
11.2	1.500	1.763	1.520	1.363	1.448	1.529
12.3	2.851	2.901	2.580	2.335	2.469	2.703

GLUCOSE MEASUREMENTS (mM)

Time (hr)	[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc
2.0	14.9	13.7	14.2	14.1	14.2	14.5
8.0	14.0	13.2	13.6	13.4	13.5	13.6
10.1	11.5	10.2	10.8	10.6	10.6	10.4
11.2	8.0	6.3	7.5	7.5	7.1	6.8
12.3	1.1	0.0	1.8	3.0	2.2	1.1

ESTIMATED GROWTH RATES (1/hr)

[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc
0.70	0.72	0.68	0.69	0.65	0.63

Table S3. Mass isotopomer distributions of 13C-labeled glucose tracers used in parallel labeling experiments with E. coli.

MEASURED MASS ISOTOPOMER DISTRIBUTIONS

	[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc
Gluc173 (M0)	90.8	90.8	90.6	90.6	0.9	1.4
Gluc173 (M1)	8.1	8.1	8.2	8.2	91.9	91.3
Gluc173 (M2)	1.2	1.1	1.2	1.2	7.3	7.3
Gluc370 (M0)	0.3	0.2	0.2	0.4	0.8	80.9
Gluc370 (M1)	81.8	81.9	81.9	81.7	81.5	15.7
Gluc370 (M2)	14.8	14.7	14.8	14.7	14.7	2.8
Gluc370 (M3)	2.7	2.7	2.7	2.7	2.7	0.5
Gluc370 (M4)	0.3	0.3	0.4	0.3	0.3	0.1
Gluc370 (M5)	0.1	0.1	0.1	0.1	0.1	0.1
Gluc370 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Gluc370 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Gluc370 (M8)	0.0	0.0	0.0	0.0	0.0	0.0

CORRECTED MASS ISOTOPOMER DISTRIBUTIONS

	[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc
Gluc173 (M0)	99.9	99.9	99.9	99.9	0.9	1.5
Gluc173 (M1)	0.0	0.0	0.0	0.0	99.1	98.5
Gluc173 (M2)	0.1	0.1	0.1	0.1	0.0	0.0
Gluc370 (M0)	0.3	0.3	0.3	0.5	1.0	99.7
Gluc370 (M1)	99.6	99.5	99.5	99.2	98.8	0.0
Gluc370 (M2)	0.0	0.0	0.0	0.0	0.0	0.0
Gluc370 (M3)	0.1	0.2	0.2	0.1	0.2	0.1
Gluc370 (M4)	0.0	0.0	0.0	0.0	0.0	0.0
Gluc370 (M5)	0.0	0.0	0.0	0.0	0.0	0.1
Gluc370 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Gluc370 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Gluc370 (M8)	0.0	0.0	0.0	0.0	0.0	0.0

MEASURED AMINO ACID FRAGMENTS

Fragment	Formula	C-atoms
Gluc173	C8H13O4	5-6
Gluc370	C17H24O8N	1-2-3-4-5

ISOTOPIC PURITY OF TRACER (% 13C)

	[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc
Isotopic Purity	99.6%	99.5%	99.5%	99.2%	99.0%	98.5%

Table S4. Mass isotopomer distributions of biomass amino acids for *E. coli* grown in parallel batch cultures on six singly labeled 13C-glucose tracers.

MEASURED MASS ISOTOPOMER DISTRIBUTIONS

	[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc
Ala232 (M0)	45.8	40.6	70.8	75.6	36.0	36.0
Ala232 (M1)	40.2	44.4	20.2	16.4	48.1	48.0
Ala232 (M2)	10.4	11.0	7.5	6.8	11.4	11.6
Ala232 (M3)	3.2	3.6	1.3	1.0	3.9	3.9
Ala232 (M4)	0.5	0.5	0.2	0.2	0.5	0.6
Ala260 (M0)	44.5	40.5	41.2	36.4	36.2	36.0
Ala260 (M1)	40.6	41.5	41.2	47.1	47.2	47.4
Ala260 (M2)	10.7	13.0	12.7	11.7	11.8	11.7
Ala260 (M3)	3.5	4.1	4.1	4.1	4.2	4.2
Ala260 (M4)	0.5	0.7	0.7	0.6	0.6	0.6
Ala260 (M5)	0.1	0.1	0.1	0.1	0.1	0.1
Gly218 (M0)	76.0	46.7	72.6	76.7	37.2	75.7
Gly218 (M1)	16.4	40.3	19.1	15.8	48.0	16.6
Gly218 (M2)	6.6	9.9	7.0	6.6	11.0	6.7
Gly218 (M3)	0.9	3.1	1.2	0.9	3.8	1.0
Gly246 (M0)	74.3	44.2	43.1	37.8	36.9	74.9
Gly246 (M1)	17.3	41.0	40.3	46.7	47.2	16.9
Gly246 (M2)	7.1	10.9	12.2	11.1	11.4	7.0
Gly246 (M3)	1.1	3.4	3.7	3.8	4.0	1.0
Gly246 (M4)	0.2	0.5	0.6	0.5	0.6	0.2
Val260 (M0)	27.3	21.5	64.0	73.2	16.9	16.8
Val260 (M1)	41.9	42.2	24.5	17.8	41.2	40.9
Val260 (M2)	22.5	26.7	9.0	7.4	31.0	31.0
Val260 (M3)	6.3	7.2	2.0	1.2	8.1	8.3
Val260 (M4)	1.7	2.1	0.4	0.2	2.4	2.5
Val260 (M5)	0.3	0.3	0.1	0.0	0.4	0.4
Val260 (M6)	0.0	0.1	0.0	0.0	0.1	0.1
Val288 (M0)	26.5	21.5	37.3	35.2	17.1	16.8
Val288 (M1)	41.9	40.6	41.2	47.1	41.0	40.7
Val288 (M2)	23.0	26.5	15.3	12.5	30.5	30.9
Val288 (M3)	6.5	8.5	4.9	4.3	8.3	8.4
Val288 (M4)	1.7	2.3	1.1	0.7	2.5	2.5
Val288 (M5)	0.3	0.5	0.2	0.1	0.5	0.5
Val288 (M6)	0.0	0.1	0.0	0.0	0.1	0.1
Val288 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Leu274 (M0)	17.1	20.2	62.7	73.2	16.9	8.6
Leu274 (M1)	36.1	40.5	25.4	18.3	40.7	28.2
Leu274 (M2)	29.9	27.6	9.2	7.0	30.8	36.0
Leu274 (M3)	12.5	8.6	2.1	1.2	8.5	19.9
Leu274 (M4)	3.4	2.4	0.4	0.2	2.5	5.4
Leu274 (M5)	0.8	0.5	0.1	0.0	0.5	1.6
Leu274 (M6)	0.1	0.1	0.0	0.0	0.1	0.2
Leu274 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Ile274 (M0)	23.2	20.4	49.2	52.3	16.2	14.9
Ile274 (M1)	39.5	40.3	33.6	33.9	39.7	38.0
Ile274 (M2)	25.5	27.4	12.6	10.3	31.2	32.1
Ile274 (M3)	8.8	8.9	3.7	2.9	9.5	11.0
Ile274 (M4)	2.4	2.4	0.8	0.5	2.8	3.2
Ile274 (M5)	0.5	0.5	0.2	0.1	0.5	0.7
Ile274 (M6)	0.1	0.1	0.0	0.0	0.1	0.1
Ile274 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Ser362 (M0)	40.4	36.4	61.7	65.5	31.5	32.5
Ser362 (M1)	39.7	41.8	24.7	22.0	45.6	45.1
Ser362 (M2)	14.6	15.8	10.9	10.2	16.4	16.1
Ser362 (M3)	5.3	6.0	2.7	2.3	6.4	6.3
Ser390 (M0)	39.2	35.0	35.8	31.2	30.4	31.8
Ser390 (M1)	39.0	39.8	39.5	44.6	44.6	44.4
Ser390 (M2)	14.8	16.9	16.4	16.1	16.5	15.9
Ser390 (M3)	5.5	6.4	6.3	6.4	6.6	6.3
Ser390 (M4)	1.2	1.6	1.6	1.4	1.5	1.4
Ser390 (M5)	0.3	0.4	0.3	0.3	0.3	0.3
Phe302 (M0)	71.6	41.5	40.2	34.4	34.4	72.5
Phe302 (M1)	19.4	41.9	41.9	48.1	47.8	18.9
Phe302 (M2)	7.7	12.7	13.5	13.2	13.3	7.4
Phe302 (M3)	1.3	4.0	4.3	4.3	4.5	1.1

CORRECTED MASS ISOTOPOMER DISTRIBUTIONS

	[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc
Ala232 (M0)	60.6	53.7	93.8	100.1	47.6	47.6
Ala232 (M1)	39.4	46.4	6.1	-0.2	52.6	52.5
Ala232 (M2)	0.0	-0.1	0.2	0.1	-0.2	0.0
Ala232 (M3)	-0.1	0.0	0.0	0.0	-0.1	-0.1
Ala232 (M4)	0.0	0.0	0.0	0.0	0.0	0.0
Ala260 (M0)	59.8	54.4	55.3	48.9	48.5	48.3
Ala260 (M1)	40.3	42.7	42.1	51.4	51.6	51.9
Ala260 (M2)	-0.2	2.8	2.4	-0.3	-0.2	-0.4
Ala260 (M3)	0.1	0.1	0.2	0.0	0.2	0.2
Ala260 (M4)	0.0	0.0	0.0	0.0	0.0	0.0
Ala260 (M5)	0.0	0.0	0.0	0.0	0.0	0.0
Gly218 (M0)	99.3	60.8	94.9	100.3	48.5	98.9
Gly218 (M1)	0.8	39.4	5.2	-0.2	51.8	1.2
Gly218 (M2)	-0.2	-0.3	-0.2	-0.1	-0.3	-0.1
Gly218 (M3)	0.0	0.0	0.0	0.0	0.0	0.0
Gly246 (M0)	98.6	58.6	57.2	50.2	48.9	99.3
Gly246 (M1)	1.3	41.0	40.5	50.4	51.3	0.7
Gly246 (M2)	0.1	0.4	2.4	-0.5	-0.2	0.0
Gly246 (M3)	0.0	0.0	0.0	-0.1	0.0	0.0
Gly246 (M4)	0.0	0.0	0.0	0.0	0.0	0.0
Val260 (M0)	37.0	29.1	86.6	99.2	22.8	22.8
Val260 (M1)	47.2	49.5	12.2	0.2	49.8	49.3
Val260 (M2)	15.6	21.3	1.2	0.6	27.5	27.7
Val260 (M3)	0.1	-0.1	0.0	0.0	-0.2	0.1
Val260 (M4)	0.1	0.1	0.0	0.0	0.1	0.1
Val260 (M5)	0.0	0.0	0.0	0.0	0.0	0.0
Val260 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Val288 (M0)	36.4	29.5	51.2	48.4	23.5	23.1
Val288 (M1)	47.7	47.8	43.1	51.9	49.8	49.5
Val288 (M2)	15.9	21.2	5.2	-0.3	26.7	27.4
Val288 (M3)	-0.1	1.4	0.4	0.0	-0.1	-0.1
Val288 (M4)	0.0	0.0	0.0	0.0	0.0	0.0
Val288 (M5)	0.1	0.1	0.1	0.0	0.1	0.2
Val288 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Val288 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Leu274 (M0)	23.4	27.7	85.9	100.3	23.1	11.8
Leu274 (M1)	43.0	48.0	13.0	-0.2	49.3	35.2
Leu274 (M2)	27.6	22.8	1.1	-0.1	27.3	38.7
Leu274 (M3)	5.9	1.3	0.1	0.0	0.1	14.1
Leu274 (M4)	0.0	0.0	0.0	0.0	0.0	-0.1
Leu274 (M5)	0.1	0.1	0.1	0.0	0.1	0.2
Leu274 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Leu274 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Ile274 (M0)	31.8	28.0	67.4	71.7	22.2	20.4
Ile274 (M1)	45.6	47.5	28.6	27.9	48.2	46.4
Ile274 (M2)	20.2	22.6	3.6	0.2	28.2	29.9
Ile274 (M3)	2.4	1.8	0.3	0.1	1.4	3.1
Ile274 (M4)	0.0	0.0	0.0	0.0	0.0	0.1
Ile274 (M5)	0.1	0.1	0.0	0.0	0.1	0.1
Ile274 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Ile274 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Ser362 (M0)	61.3	55.1	94.2	100.2	47.6	49.2
Ser362 (M1)	39.1	44.1	5.8	-0.2	52.4	51.1
Ser362 (M2)	-0.4	0.7	0.0	0.0	0.1	-0.3
Ser362 (M3)	0.0	0.0	-0.1	0.0	-0.1	-0.1
Ser390 (M0)	61.2	54.5	55.9	48.7	47.4	49.6
Ser390 (M1)	39.0	42.5	41.7	52.0	52.5	51.3
Ser390 (M2)	-0.3	2.9	2.3	-0.6	0.0	-0.8
Ser390 (M3)	0.1	0.1	0.2	0.0	0.1	-0.1
Ser390 (M4)	0.0	0.0	0.0	0.0	0.0	0.0
Ser390 (M5)	0.0	0.0	0.0	0.0	0.0	0.0
Phe302 (M0)	99.1	57.1	55.2	47.4	47.4	100.5
Phe302 (M1)	0.6	42.1	42.6	53.1	52.7	-0.3
Phe302 (M2)	0.3	0.8	2.0	-0.3	-0.1	0.1
Phe302 (M3)	0.0	0.0	0.2	-0.1	0.1	-0.2

AVERAGE CARBON LABELING (% 13C)

	[1]Glc	[2]Glc	[3]Glc	[4]Glc	[5]Glc	[6]Glc	SUM
Ala232	19.7	23.1	3.2	-0.1	26.1	26.3	98.4
Ala260	13.4	16.2	15.8	17.0	17.2	17.2	96.9
Gly218	0.8	39.4	5.2	-0.2	51.8	1.2	98.2
Gly246	0.7	20.9	22.6	24.7	25.4	0.4	94.8
Val260	19.8	23.1	3.7	0.4	26.1	26.4	99.4
Val288	15.9	19.0	11.0	10.3	20.7	20.9	97.9
Leu274	23.2	19.7	3.1	0.0	21.0	31.2	98.2
Ile274	18.7	19.7	7.4	5.8	21.8	23.2	96.6
Ser362	19.2	22.8	2.9	-0.1	26.3	25.3	96.3
Ser390	12.9	16.2	15.6	16.9	17.6	16.5	95.7
Phe302	0.6	21.9	23.3	26.2	26.2	-0.1	98.2
Phe308	12.6	15.9	15.6	9.4	22.6	22.3	98.4
Asp302	12.3	17.6	14.8	16.5	19.6	13.9	94.6
Asp390	17.8	17.0	9.6	9.1	18.7	21.1	93.3
Asp418	15.0	15.5	13.4	15.0	16.5	16.9	92.4
Glu330	21.0	21.7	3.3	0.0	24.3	27.9	98.3
Glu432	18.6	19.5	7.2	5.4	21.5	23.4	95.6
Tyr302	0.3	21.6	23.9	26.1	26.1	0.5	98.5

MEASURED AMINO ACID FRAGMENTS

Fragment	Formula	C-atoms
Ala232	C10H26ONSi2	2-3
Ala260	C11H26O2NSi2	1-2-3
Gly218	C9H24ONSi2	2
Gly246	C10H24O2NSi2	1-2
Val260	C12H30ONSi2	2-3-4-5
Val288	C13H30O2NSi2	1-2-3-4-5
Leu274	C13H32ONSi2	2-3-4-5-6
Ile274	C13H32ONSi2	2-3-4-5-6
Ser362	C16H40O2NSi3	2-3
Ser390	C17H40O3NSi3	1-2-3
Phe302	C14H32O2NSi2	1-2
Phe308	C16H30ONSi2	2-3-4-5-6-7-8-9
Asp302	C14H32O2NSi2	1-2
Asp390	C17H40O3NSi3	2-3-4
Asp418	C18H40O4NSi3	1-2-3-4
Glu330	C16H36O2NSi2	2-3-4-5
Glu432	C19H42O4NSi3	1-2-3-4-5
Tyr302	C14H32O2NSi2	1-2

Phe308 (M0)	20.4	14.6	4.5	17.5	4.0	4.3	Phe308 (M0)	28.9	20.6	6.4	24.8	5.6	6.0
Phe308 (M1)	37.8	34.0	49.0	58.6	21.6	22.0	Phe308 (M1)	44.7	41.7	66.8	75.0	28.6	29.0
Phe308 (M2)	27.2	30.2	30.2	16.6	38.0	38.2	Phe308 (M2)	22.6	28.3	23.0	0.0	44.3	44.4
Phe308 (M3)	10.5	14.5	11.8	6.0	26.3	25.8	Phe308 (M3)	3.5	7.8	3.3	0.2	21.5	20.7
Phe308 (M4)	3.0	5.1	3.5	1.0	7.5	7.3	Phe308 (M4)	0.1	1.3	0.4	-0.1	-0.1	-0.2
Phe308 (M5)	0.6	1.3	0.8	0.2	2.1	1.9	Phe308 (M5)	0.0	0.0	0.0	0.0	-0.1	-0.2
Phe308 (M6)	0.1	0.3	0.1	0.0	0.3	0.3	Phe308 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Phe308 (M7)	0.1	0.1	0.0	0.0	0.1	0.1	Phe308 (M7)	0.1	0.0	0.0	0.0	0.0	0.0
Phe308 (M8)	0.2	0.1	0.1	0.0	0.1	0.1	Phe308 (M8)	0.2	0.1	0.1	0.0	0.1	0.2
Asp302 (M0)	55.9	47.4	51.9	48.6	44.1	52.8	Asp302 (M0)	77.0	65.2	71.5	66.9	60.7	72.9
Asp302 (M1)	30.3	37.6	33.7	37.0	40.4	33.3	Asp302 (M1)	21.1	34.1	27.2	33.0	39.1	26.3
Asp302 (M2)	10.9	11.6	11.3	11.1	11.8	10.8	Asp302 (M2)	1.7	0.5	1.2	0.0	0.1	0.7
Asp302 (M3)	2.9	3.5	3.2	3.3	3.7	3.1	Asp302 (M3)	0.1	0.1	0.1	0.1	0.1	0.1
Asp390 (M0)	33.7	33.6	46.6	46.4	29.7	27.4	Asp390 (M0)	52.5	52.3	72.7	72.4	46.3	42.7
Asp390 (M1)	39.0	40.4	33.1	34.3	43.6	43.0	Asp390 (M1)	41.9	44.2	25.9	27.9	51.2	51.6
Asp390 (M2)	18.1	17.4	14.2	13.4	17.7	19.3	Asp390 (M2)	5.3	3.4	1.4	-0.4	2.4	5.4
Asp390 (M3)	6.9	6.6	4.8	4.6	6.9	7.7	Asp390 (M3)	0.3	0.0	0.1	0.0	0.0	0.3
Asp390 (M4)	1.9	1.7	1.1	1.0	1.7	2.1	Asp390 (M4)	0.0	0.0	-0.1	0.0	0.0	0.0
Asp390 (M5)	0.4	0.4	0.2	0.2	0.4	0.5	Asp390 (M5)	0.0	0.0	0.0	0.0	0.0	0.0
Asp418 (M0)	31.1	29.9	35.6	32.9	26.3	26.1	Asp418 (M0)	49.1	47.2	56.2	52.0	41.6	41.2
Asp418 (M1)	38.4	38.8	34.8	35.0	42.2	41.8	Asp418 (M1)	42.6	43.9	34.4	36.2	51.1	50.7
Asp418 (M2)	19.8	20.3	19.5	21.2	20.4	20.5	Asp418 (M2)	7.8	8.5	8.9	11.7	7.0	7.4
Asp418 (M3)	7.7	7.9	7.3	7.7	8.1	8.4	Asp418 (M3)	0.4	0.3	0.4	0.0	0.2	0.6
Asp418 (M4)	2.4	2.4	2.3	2.5	2.4	2.5	Asp418 (M4)	0.2	0.1	0.1	0.1	0.1	0.1
Asp418 (M5)	0.6	0.6	0.5	0.6	0.5	0.6	Asp418 (M5)	0.0	0.0	0.0	0.0	0.0	0.0
Asp418 (M6)	0.1	0.1	0.1	0.1	0.1	0.1	Asp418 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Glu330 (M0)	24.9	22.9	62.1	70.6	18.8	15.3	Glu330 (M0)	35.4	32.5	88.2	100.2	26.7	21.6
Glu330 (M1)	40.3	41.2	25.4	19.9	40.8	38.2	Glu330 (M1)	46.5	48.6	10.7	-0.4	49.7	47.4
Glu330 (M2)	24.1	25.4	9.5	7.7	28.7	31.7	Glu330 (M2)	17.0	18.5	1.0	0.1	23.4	28.8
Glu330 (M3)	7.9	7.8	2.3	1.4	8.6	10.7	Glu330 (M3)	0.9	0.3	0.1	0.1	0.1	1.9
Glu330 (M4)	2.3	2.3	0.5	0.3	2.6	3.3	Glu330 (M4)	0.2	0.1	0.1	0.0	0.1	0.2
Glu330 (M5)	0.4	0.4	0.1	0.0	0.5	0.7	Glu330 (M5)	0.0	0.0	0.0	0.0	0.0	0.0
Glu330 (M6)	0.1	0.1	0.0	0.0	0.1	0.1	Glu330 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Glu432 (M0)	20.1	17.9	42.7	45.7	14.4	13.1	Glu432 (M0)	32.2	28.7	68.3	73.0	23.0	20.9
Glu432 (M1)	36.2	36.7	33.5	34.1	36.1	33.9	Glu432 (M1)	45.4	47.4	27.9	27.1	48.6	45.9
Glu432 (M2)	26.5	28.0	15.9	13.9	30.8	31.5	Glu432 (M2)	19.8	22.0	3.3	-0.4	26.9	29.3
Glu432 (M3)	11.6	11.8	5.8	4.8	12.6	14.2	Glu432 (M3)	2.3	1.7	0.4	0.1	1.2	3.4
Glu432 (M4)	4.0	4.1	1.6	1.2	4.6	5.4	Glu432 (M4)	0.1	0.1	0.1	0.0	0.2	0.3
Glu432 (M5)	1.2	1.1	0.4	0.3	1.2	1.5	Glu432 (M5)	0.2	0.1	0.1	0.0	0.1	0.2
Glu432 (M6)	0.3	0.3	0.1	0.0	0.3	0.4	Glu432 (M6)	0.0	0.0	0.0	0.0	0.0	0.0
Glu432 (M7)	0.1	0.1	0.0	0.0	0.1	0.1	Glu432 (M7)	0.0	0.0	0.0	0.0	0.0	0.0
Tyr302 (M0)	71.6	41.4	39.5	34.2	34.4	71.6	Tyr302 (M0)	99.3	57.3	54.7	47.4	47.7	99.3
Tyr302 (M1)	19.2	41.4	41.5	47.3	47.2	19.2	Tyr302 (M1)	0.4	41.8	42.6	52.4	52.3	0.4
Tyr302 (M2)	7.5	12.5	13.9	13.1	13.1	7.6	Tyr302 (M2)	0.1	0.7	2.6	-0.1	-0.1	0.3
Tyr302 (M3)	1.3	3.9	4.3	4.5	4.5	1.4	Tyr302 (M3)	0.1	0.1	0.0	0.1	0.1	0.0
Tyr302 (M4)	0.4	0.8	0.9	0.9	0.8	0.3	Tyr302 (M4)	0.2	0.1	0.1	0.2	0.1	0.1

Table S5. Results of 13C-MFA and COMPLETE-MFA for *E. coli* grown in parallel batch cultures on six singly labeled 13C-glucose tracers.

Shown are the estimated net and exchange fluxes (normalized to glucose uptake rate of 100).
Accurate 95% confidence intervals of fluxes (LB95 = lower bound, UB95 = upper bound) were determined by evaluating the sensitivity of the minimized SSR to flux variations (Antoniewicz et al., 2006)

	Data set :	[1-13C]Glucose			[2-13C]Glucose			[3-13C]Glucose			[4-13C]Glucose			[5-13C]Glucose			[6-13C]Glucose			COMPLETE-MFA		
	SSR :	13			23			18			43			41			44			257		
	Fit Statistically Accepted :	Yes			Yes			Yes			Yes			Yes			Yes			Yes		
INTRACELLULAR FLUXES																						
Flux No.	Reaction	Best Fit	LB95	UB95	Best Fit	LB95	UB95	Best Fit	LB95	UB95	Best Fit	LB95	UB95	Best Fit	LB95	UB95	Best Fit	LB95	UB95	Best Fit	LB95	UB95
1	Gluc.Ext + PEP -> G6P + Pyr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2	G6P <=> F6P (net)	68.6	65.6	75.9	71.7	68.7	74.9	74.3	72.1	76.1	59.6	-45.0	78.3	75.9	69.9	83.2	69.8	55.1	82.3	69.6	68.2	71.3
3	F6P + ATP -> FBP + ADP	81.6	80.0	84.3	83.4	80.5	84.3	83.1	82.2	84.5	77.9	39.0	84.2	83.6	81.0	86.3	82.7	76.5	86.7	81.6	81.1	82.2
4	FBP <=> DHAP + GAP (net)	81.6	80.0	84.3	83.4	80.5	84.3	83.1	82.2	84.5	77.9	39.0	84.2	83.6	81.0	86.3	82.7	76.5	86.7	81.6	81.1	82.2
5	DHAP <=> GAP (net)	81.6	80.0	84.3	83.4	80.5	84.3	83.1	82.2	84.5	77.9	39.0	84.2	83.6	81.0	86.3	82.7	76.5	86.7	81.6	81.1	82.2
6	GAP + NAD + ADP + Pi <=> 3PG + ATP + NADH (net)	168.9	166.5	171.9	171.0	167.7	172.3	171.0	169.3	173.9	166.1	129.1	172.8	171.1	168.1	173.7	170.2	164.0	174.3	169.2	168.4	169.8
7	3PG <=> PEP (net)	158.0	152.6	163.4	157.7	153.6	159.6	157.9	155.2	163.1	153.4	118.4	160.9	157.5	153.8	160.6	157.8	151.1	162.6	155.8	154.7	156.6
8	PEP + ADP -> Pyr + ATP	27.1	20.3	34.3	29.8	26.0	34.9	34.8	18.4	40.6	30.3	0.0	38.5	29.2	25.2	34.8	28.8	21.1	35.4	28.2	26.9	30.2
9	G6P + NADP -> 6PG + NADPH	29.8	22.5	32.8	26.7	23.4	29.8	24.1	22.5	26.3	38.9	20.1	144.0	22.5	15.1	28.5	28.6	16.0	43.4	28.8	27.1	30.3
10	6PG + NADP -> Ru5P + CO2 + NADPH	28.8	21.8	31.8	26.6	23.4	28.8	22.1	20.1	24.6	36.2	16.7	144.0	20.9	13.5	27.0	28.6	16.0	42.3	27.2	25.4	28.7
11	Ru5P <=> X5P (net)	13.5	8.7	15.7	12.2	9.6	13.9	9.3	8.0	11.1	18.9	5.5	92.4	8.3	3.1	12.6	13.5	4.7	22.8	12.6	11.3	13.6
12	Ru5P <=> R5P (net)	15.2	13.0	16.5	14.4	13.6	15.2	12.8	11.7	13.7	17.3	11.1	51.9	12.5	10.3	14.6	15.1	11.1	19.6	14.6	14.1	15.1
13	X5P <=> GAP + E-C2 (net)	13.5	8.7	15.7	12.2	9.6	13.9	9.3	8.0	11.1	18.9	5.5	92.4	8.3	3.1	12.6	13.5	4.7	22.8	12.6	11.3	13.6
14	F6P <=> E4P + E-C2 (net)	-5.3	-6.5	-2.8	-4.7	-5.6	-3.3	-3.3	-4.3	-2.6	-8.1	-41.8	-1.3	-2.7	-4.9	0.0	-5.4	-10.1	-0.9	-4.9	-5.4	-4.3
15	S7P <=> R5P + E-C2 (net)	-8.2	-9.2	-5.8	-7.5	-8.3	-6.3	-6.0	-6.8	-5.4	-10.8	-47.1	-4.2	-5.6	-7.6	-3.0	-8.2	-12.7	-3.9	-7.7	-8.2	-7.1
16	F6P <=> GAP + E-C3 (net)	-8.2	-9.2	-5.8	-7.5	-8.3	-6.3	-6.0	-6.8	-5.4	-10.8	-47.1	-4.2	-5.6	-7.6	-3.0	-8.2	-12.7	-3.9	-7.7	-8.2	-7.1
17	S7P <=> E4P + E-C3 (net)	8.2	5.8	9.2	7.5	6.3	8.3	6.0	5.4	6.8	10.8	4.2	47.1	5.6	3.0	7.6	8.2	3.9	12.7	7.7	7.1	8.2
18	6PG -> KDPG	1.0	0.1	1.9	0.1	0.0	3.1	2.1	1.0	2.8	2.7	0.0	34.3	1.7	0.6	3.1	0.0	0.0	2.8	1.6	1.3	2.0
19	KDPG -> GAP + Pyr	1.0	0.1	1.9	0.1	0.0	3.1	2.1	1.0	2.8	2.7	0.0	34.3	1.7	0.6	3.1	0.0	0.0	2.8	1.6	1.3	2.0
20	Pyr + NAD -> AcCoA + CO2 + NADH	107.4	99.2	116.1	109.7	103.0	114.2	117.1	105.7	125.7	113.8	87.0	124.2	110.4	103.5	115.9	108.4	100.5	116.0	109.5	106.9	111.6
21	AcCoA + OAC -> Cit	16.4	12.4	22.1	17.6	14.1	19.4	20.7	16.6	29.5	18.8	5.7	30.3	17.5	15.4	19.0	16.4	14.2	18.5	16.5	15.8	16.9
22	Cit <=> ICit (net)	16.4	12.4	22.1	17.6	14.1	19.4	20.7	16.6	29.5	18.8	5.7	30.3	17.5	15.4	19.0	16.4	14.2	18.5	16.5	15.8	16.9
23	ICit + NADP <=> AKG + CO2 + NADPH (net)	13.1	8.2	20.6	17.6	12.4	19.4	16.5	13.3	27.4	15.3	5.7	30.2	17.5	14.9	19.0	15.0	12.8	17.8	15.9	15.0	16.6
24	AKG + NAD -> SucCoA + CO2 + NADH	4.7	0.0	12.4	9.4	4.0	11.1	8.4	5.0	19.7	7.4	0.0	24.2	9.1	6.8	10.4	6.6	4.7	9.1	7.6	6.8	8.2
25	SucCoA + ADP + Pi <=> Suc + ATP (net)	0.9	-4.0	8.7	5.8	0.3	7.6	4.9	1.3	16.4	3.9	-4.3	21.7	5.5	3.2	6.7	2.9	1.2	5.3	4.0	3.1	4.6
26	Suc + FAD <=> Fum + FADH2 (net)	7.9	4.1	13.7	9.4	5.9	11.1	12.6	8.4	21.9	10.9	0.0	24.3	9.1	7.5	10.4	8.0	6.5	10.1	8.1	7.6	8.6
27	Fum <=> Mal (net)	10.9	6.9	16.6	12.2	8.8	14.0	15.4	11.2	24.5	13.6	2.0	26.4	12.0	10.2	13.3	10.9	9.2	13.1	11.0	10.4	11.5
28	Mal + NAD <=> OAC + NADH (net)	14.2	8.6	18.9	12.2	10.6	14.5	19.6	6.0	23.4	17.1	-25.2	26.4	12.0	10.6	13.5	12.4	8.2	14.8	11.5	10.9	12.1
29	ICit -> Glyox + Suc	3.3	0.5	6.6	0.0	0.0	1.9	4.2	0.0	5.9	3.5	0.0	9.3	0.0	0.0	0.8	1.4	0.2	2.7	0.5	0.1	0.9
30	AcCoA + Glyox -> Mal	3.3	0.5	6.6	0.0	0.0	1.9	4.2	0.0	5.9	3.5	0.0	9.3	0.0	0.0	0.8	1.4	0.2	2.7	0.5	0.1	0.9
31	Mal + NADP -> Pyr + CO2 + NADPH	0.0	0.0	4.7	0.0	0.0	2.4	0.0	0.0	14.4	0.0	0.0	30.9	0.0	0.0	2.2	0.0	0.0	4.3	0.0	0.0	0.9
32	Mal + NAD -> Pyr + CO2 + NADH	0.0	0.0	4.7	0.0	0.0	2.4	0.0	0.0	14.4	0.0	0.0	35.6	0.0	0.0	2.2	0.0	0.0	4.3	0.0	0.0	0.9
33	PEP + CO2 -> OAC + Pi	29.4	21.2	37.6	22.0	19.7	25.5	17.3	15.4	36.7	23.3	13.8	298.4	22.3	20.2	24.6	23.4	19.1	27.9	22.4	21.1	23.5
34	OAC + ATP -> PEP + CO2 + ADP	4.7	0.0	8.4	0.0	0.0	3.6	0.0	0.0	7.0	5.8	0.0	284.3	0.0	0.0	2.3	0.4	0.0	2.1	0.9	0.0	2.1
35	AcCoA + ADP + Pi <=> Ac + ATP (net)	66.0	55.9	76.0	65.6	55.8	75.6	66.3	56.3	76.1	66.2	55.6	77.0	66.0	55.9	75.9	66.0	55.9	76.1	65.9	61.9	70.0
36	AKG + NADPH + NH3 -> Glu + NADP	54.2	47.1	61.4	50.1	47.2	54.7	49.0	43.0	53.9	47.8	32.3	59.4	50.8	47.2	55.6	51.8	45.8	57.5	50.4	49.0	52.1
37	Glu + ATP + NH3 -> Gln + ADP + Pi	5.3	4.8	5.8	5.2	4.9	5.6	5.1	4.4	5.5	4.9	3.3	6.1	5.3	4.9	5.8	5.2	4.7	5.8	5.2	5.1	5.4
38	Glu + 2 NADPH + ATP -> Pro + 2 NADP + ADP + Pi	1.7	1.5	1.8	1.6	1.5	1.8	1.6	1.4	1.7	1.5	1.0	1.9	1.6	1.5	1.8	1.6	1.5	1.8	1.6	1.6	1.7
39	Glu + CO2 + Gln + NADPH + Asp + AcCoA + 5 ATP -> Arg + AKG + NADP + Fum + A	2.2	2.0	2.4	2.2	2.0	2.4	2.1	1.8	2.3	2.1	1.4	2.6	2.2	2.0	2.4	2.2	1.9	2.4	2.2	2.1	2.2
40	OAC + Glu -> Asp + AKG	19.8	13.1	26.4	13.9	13.1	15.2	13.6	12.0	16.4	13.3	9.0	16.5	14.2	13.1	15.5	16.3	12.8	20.7	14.0	13.7	14.5
41	Asp + NH3 + 2 ATP -> Asn + 2 ADP + 2 Pi	1.8	1.6	2.0	1.8	1.7	1.9	1.7	1.5	1.9	1.7	1.1	2.1	1.8	1.7	2.0	1.8	1.6	2.0	1.8	1.7	1.8
42	Pyr + Glu -> Ala + AKG	3.8	3.5	4.2	3.7	3.5	4.1	3.7	3.2	4.0	3.6	2.4	4.4	3.8	3.5	4.2	3.8	3.4	4.2	3.8	3.7	3.9
43	3PG + Glu + NAD -> Ser + NADH + AKG + Pi	6.1	3.6	9.4	8.6	8.1	9.4	8.4	6.8	9.1	8.2	5.6	10.2	8.7	8.1	9.6	7.6	5.2	9.5	8.7	8.4	9.0
44	Ser + THF <=> Gly + MEETHF (net)	2.2	-0.1	5.3	4.8	4.6	5.3	4.7	3.5	5.1	4.6	3.1	5.7	4.9	4.6	5.4	3.8	1.7	5.3	4.9	4.7	5.0
45	Gly + THF + NAD <=> CO2 + MEETHF + NH3 + NADH (net)	3.1	0.3	5.8	0.4	0.4	0.6	0.4	0.3	1.3	0.4	0.2	0.8	0.4	0.4	0.5	1.5	0.3	3.5	0.4	0.4	0.4
46	Thr + NAD -> Gly + AcCoA + NADH	5.5	0.0	10.8	0.0	0.0	0.4	0.0	0.0	1.8	0.0	0.0	0.7	0.0	0.0	0.2	2.2	0.0	6.2	0.0	0.0	0.1
47	Ser + AcCoA + SO4 + 3 ATP + 4 NADPH -> Cys + Ac + 4 NADP + 3 ADP + 3 Pi	1.8	1.7	2.0	1.8	1.7	2.0	1.7	1.5	1.9	1.7	1.2	2.1	1.8	1.7	2.0	1.8	1.6	2.0	1.8	1.7	1.9
48	Asp + Pyr + Glu + 2 NADPH + ATP + SucCoA -> LL-DAP + AKG + 2 NADP + ADP + P	2.6	2.3	2.8	2.5	2.4	2.7	2.4	2.1	2.6	2.4	1.6	3.0	2.5	2.4	2.8	2.5	2.3	2.8	2.5	2.4	2.6
49	LL-DAP -> Lys + CO2	2.6	2.3	2.8	2.5	2.4	2.7	2.4	2.1	2.6	2.4	1.6	3.0	2.5	2.4	2.8	2.5	2.3	2.8	2.5	2.4	2.6
50	Asp + 2 NADPH + 2 ATP -> Thr + 2 NADP + 2 ADP + 2 Pi	9.6	3.7	15.3	4.0	3.7	4.5	3.9	3.4	5.9	3.8	2.6	5.1	4.0	3.7	4.4	6.2	3.6	10.3	4.0	3.9	4.1
51	Asp + METHF + Cys + 2 NADPH + ATP + SucCoA -> Met + Pyr + 2 NADP + ADP + Pi	1.1	1.0	1.3	1.1	1.1	1.2	1.1	1.0	1.2	1.1	0.7	1.3	1.1	1.1	1.2	1.1	1.0	1.3	1.1	1.1	1.2
52	2 Pyr + NADPH + Glu -> Val + CO2 + NADP + AKG	3.2	2.9	3.5	3.1	2.9	3.4	3.0	2.6	3.3	2.9	2.0	3.7	3.1	2.9	3.4	3.1	2.8	3.4	3.1	3.0	3.2
53	2 Pyr + AcCoA + Glu + NADPH + NAD -> Leu + 2 CO2 + AKG + NADP + NADH	3.4	3.1	3.7	3.3	3.1	3.6	3.2	2.8	3.5	3.1	2.1	3.9	3.3	3.1	3.6	3.3	3.0	3.7	3.3	3.2	3.4
54	Thr + Pyr + Glu + NADPH -> Ile + CO2 + AKG + NADP + NH3	2.2	2.0	2.4	2.1	2.0	2.3	2.1	1.8	2.2	2.0	1.4	2.5	2.1	2.0	2.4	2.1	1.9	2.4	2.1	2.1	2.2
55	E4P + 2 PEP + Glu + NADPH + ATP -> Phe + CO2 + AKG + NADP + ADP + 4 Pi	1.4	1.3	1.5	1.4	1.3	1.5	1.3	1.2	1.4	1.3	0.9	1.6	1.4	1.3	1.5	1.4	1.2	1.5	1.4	1.3	1.4
56	E4P + 2 PEP + Glu + NADPH + NAD + ATP -> Tyr + CO2 + AKG + NADP + NADH + AD	1.0	0.9	1.1	1.0	0.9	1.1	1.0	0.9	1.1	1.0	0.6	1.2	1.0	0.9	1.1	1.0	0.9	1.1	1.0	1.0	1.0
57	E4P + 2 PEP + R5P + Ser + Gln + NADPH + 3 ATP -> Trp + CO2 + Pyr + GAP + Gl	0.4	0.4	0.5	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.3	0.5	0.4	0.4	0.5	0.4	0.4	0.5	0.4	0.4	0.4
58	R5P + FTHF + Gln + Asp + 5 ATP + 2 NAD -> His + 2 NADH + AKG + Fum + 5 ADP	0.7	0.6	0.8	0.7	0.7	0.8	0.7	0.6	0.7	0.7	0.4	0.8	0.7	0.7	0.8	0.7	0.6	0.8	0.7	0.7	0.7
59	MEETHF + NADH -> METHF + NAD	1.1	1.0	1.3	1.1	1.1	1.2	1.1	1.0	1.2	1.1	0.7	1.3	1.1	1.1	1.2	1.1	1.0	1.3	1.1	1.1	1.2
60	MEETHF + NADP -> FTHF + NADPH	0.7	0.6	0.8	0.7	0.7	0.8	0.7	0.6	0.7	0.7	0.4	0.8	0.7	0.7	0.8	0.7	0.6	0.8	0.7	0.7	0.7
61	NADH + 0.5 O2 + 2 ADP + 2 Pi -> 2 ATP + NAD	250.9	213.6	282.1	268.5	243.8	279.1	276.3	252.4	322.0	294.9	193.8	519.2	257.3	230.7	276.0	260.6	227.6	294.9	264.4	257.0	269.2
62	FADH2 + 0.5 O2 + ADP + Pi -> ATP + FAD	7.9	4.1	13.7	9.4	5.9	11.1	12.6	8.4	21.9	10.9	0.0	24.3	9.1	7.5	10.4	8.0	6.5	10.1	8.1	7.6	8.6
63	NADH + NADP <=> NADPH + NAD (net)	75.1	44.2	112.2	58.6	51.0	76.4	64.0	30.2	79.7	33.0	-230.4	78.3	70.4	52.1	94.8	64.2	26.6	98.8	58.3	54.6	64.4
64	ATP -> ATP.Ext	367.3	242.7	478.5	435.0	340.9	480.3	465.5	378.7	610.0	497.5	187.9	>1000	407.4	312.8	473.8	406.2	295.4	521.0	420.0	389.7	441.3
65	Ac -> Ac.Ext	70.0	60.3	79.8	69.5	60.2	79.2	70.2	60.4	79.8	70.0	60.2	79.8	70.0	60.3	79.7	70.0	60.3	79.7	69.9	66.0	73.8
66	CO2 -> CO2.Ext	147.5	129.5	162.9	156.6	144.7	161.7	161.8	149.5	186.7	169.8	117.8	248.8	151.1	139.1	159.6	152.2	137.8	167.6	154.0	150.9	156.1
67	O2.Ext -> O2	129.4	110.0	146.1	138.9	125.5	144.7	144.5	131.3	171.4	152.9	96.9	237.2	133.2	119.7	142.6	134.3	118.1	151.4	136.3	132.6	138.6
68	NH3.Ext -> NH3	54.8	49.8	60.2	53.4	50.3	58.3	52.3	45.7	56.6	50.9	34.5	63.3	54.2	50.3	59.3	54.0	48.2	59.7	53.7	52.3	55.6
69	SO4.Ext -> SO4	1.8	1.7	2.0	1.8	1.7	2.0	1.7	1.5	1.9	1.7	1.2	2.1	1.8	1.7	2.0	1.8	1.6	2.0	1.8	1.7	1.9
70	0.488 Ala + 0.281 Arg + 0.229 Asn + 0.229 Asp + 0.087 Cys + 0.25 Glu + 0.25	7.9	7.1	8.6	7.7	7.2	8.4	7.5	6.6	8.1	7.3	5.0	9.1	7.8	7.2	8.5	7.8	6.9	8.6	7.7	7.5	8.0
71	CO2.unlabeled + CO2 -> CO2 + CO2.out	34.2	0.0	98.9	123.7	0.0	368.4	0.0	0.0	57.4	5.2	0.0	65.7	415.4	0.0	Inf	0.5	0.0	Inf	47.8	42.2	54.8

EXCHANGE FLUXES																					
2	G6P <=> F6P (exch)	233.0	0.0	Inf	184.0	31.4	>1000	4.1	0.0	38.0	11.0	0.0	Inf	>1000	0.0	Inf	177.4	0.0	Inf	394.6	133.8	Inf
4	FBP <=> DHAP + GAP (exch)	203.1	0.0	Inf	157.4	0.0	Inf	194.2	0.0	Inf	185.6	0.0	Inf	27.1	0.0	Inf	113.3	0.0	Inf	78.2	0.0	Inf
5	DHAP <=> GAP (exch)	47.2	0.0	Inf	157.4	0.0	Inf	148.7	0.0	Inf	142.1	0.0	Inf	27.1	0.0	Inf	183.1	0.0	Inf	157.3	0.0	Inf
6	GAP + NAD + ADP + Pi <=> 3PG + ATP + NADH (exch)	2.3	0.0	Inf	>1000	0.0	Inf	43.7	0.0	Inf	41.2	0.0	Inf	583.4	0.0	Inf	2.3	0.0	Inf	1.5	0.0	Inf
7	3PG <=> PEP (exch)	0.0	0.0	Inf	Inf	0.0	Inf	1.7	0.0	Inf	1.7	0.0	Inf	>1000	0.0	Inf	0.0	0.0	Inf	0.0	0.0	Inf
11	Ru5P <=> X5P (exch)	59.4	0.0	Inf	54.6	2.8	Inf	104.1	6.3	Inf	99.9	0.0	Inf	>1000	0.0	Inf	88.2	0.0	Inf	97.9	16.5	Inf
12	Ru5P <=> R5P (exch)	11.0	0.0	Inf	336.9	0.0	Inf	523.2	3.3	Inf	499.8	0.0	Inf	212.0	0.0	Inf	147.3	0.0	Inf	1.2	0.0	Inf
13	X5P <=> GAP + E-C2 (exch)	112.1	0.0	Inf	54.6	2.8	Inf	13.1	6.2	Inf	12.9	0.0	Inf	>1000	0.0	Inf	18.5	0.0	Inf	42.5	16.3	Inf
14	F6P <=> E4P + E-C2 (exch)	1.1	0.0	10.2	5.2	2.7	7.7	2.3	0.6	4.2	6.0	0.0	Inf	>1000	4.5	Inf	110.8	0.0	Inf	2.1	0.4	3.5
15	S7P <=> R5P + E-C2 (exch)	13.4	0.0	Inf	0.0	0.0	Inf	2.1	1.2	Inf	0.0	0.0	Inf	339.3	0.0	Inf	116.2	0.0	Inf	5.9	1.0	Inf
16	F6P <=> GAP + E-C3 (exch)	0.0	0.0	Inf	48.0	0.0	237.1	>1000	0.0	Inf	>1000	0.0	Inf	2.1	0.0	97.5	81.6	0.0	Inf	16.1	1.5	47.3
17	S7P <=> E4P + E-C3 (exch)	6.4	0.0	Inf	426.4	0.0	Inf	106.8	1.3	Inf	101.9	0.0	Inf	241.4	0.0	Inf	165.9	0.0	Inf	11.7	1.1	Inf
22	Cit <=> ICit (exch)	192.2	0.0	Inf	157.4	0.0	Inf	279.7	0.0	Inf	267.2	0.0	Inf	27.1	0.0	Inf	42.1	0.0	Inf	62.4	0.0	Inf
23	ICit + NADP <=> AKG + CO2 + NADPH (exch)	256.0	0.0	Inf	111.2	0.0	Inf	653.5	0.0	Inf	623.5	0.0	Inf	>1000	0.0	Inf	190.0	0.0	Inf	0.0	0.0	Inf
25	SucCoA + ADP + Pi <=> Suc + ATP (exch)	38.3	0.0	Inf	157.4	0.0	Inf	191.0	0.0	Inf	182.4	0.0	Inf	27.1	0.0	Inf	166.6	0.0	Inf	117.2	0.0	Inf
26	Suc + FAD <=> Fum + FADH2 (exch)	57.0	0.0	Inf	157.4	0.0	Inf	2.2	0.0	Inf	2.1	0.0	Inf	27.1	0.0	Inf	106.3	0.0	Inf	149.4	0.0	Inf
27	Fum <=> Mal (exch)	463.1	74.1	Inf	>1000	35.4	Inf	>1000	30.3	Inf	>1000	13.1	Inf	611.8	48.0	Inf	138.8	51.1	Inf	>1000	57.4	Inf
28	Mal + NAD <=> OAC + NADH (exch)	140.6	73.2	Inf	53.7	38.5	64.3	17.7	5.4	Inf	24.5	0.0	Inf	63.6	49.8	Inf	131.6	50.2	Inf	62.2	55.7	67.4
35	AcCoA + ADP + Pi <=> Ac + ATP (exch)	234.3	0.0	Inf	228.2	0.0	Inf	109.5	0.0	Inf	104.6	0.0	Inf	95.4	0.0	Inf	108.4	0.0	Inf	51.1	0.0	Inf
44	Ser + THF <=> Gly + MEETHF (exch)	0.0	0.0	0.2	2.4	0.9	4.6	35.5	0.0	Inf	38.6	0.0	Inf	0.3	0.0	2.8	0.0	0.0	0.1	1.1	0.2	2.3
45	Gly + THF + NAD <=> CO2 + MEETHF + NH3 + NADH (exch)	238.5	0.0	Inf	0.0	0.0	0.3	0.0	0.0	1.4	0.0	0.0	9.1	0.0	0.0	0.2	449.8	0.0	Inf	0.1	0.1	0.2
63	NADH + NADP <=> NADPH + NAD (exch)	156.3	0.0	Inf	375.5	0.0	Inf	15.9	0.0	Inf	30.8	0.0	Inf	298.4	0.0	Inf	101.9	0.0	Inf	60.5	0.0	Inf
FRACTIONALABELING OF AMINO ACIDS (G-VALUES)																					
1	Fractional labeling of Ala (data set #1)	99%	98%	100%																99%	98%	100%
2	Fractional labeling of Gly (data set #1)	4%	3%	100%																100%	62%	100%
3	Fractional labeling of Val (data set #1)	99%	98%	100%																99%	98%	100%
4	Fractional labeling of Leu (data set #1)	99%	98%	100%																98%	98%	99%
5	Fractional labeling of Ile (data set #1)	99%	98%	100%																99%	98%	100%
6	Fractional labeling of Ser (data set #1)	97%	96%	99%																98%	96%	99%
7	Fractional labeling of Phe (data set #1)	99%	98%	100%																99%	98%	100%
8	Fractional labeling of Asp (data set #1)	99%	97%	100%																98%	97%	98%
9	Fractional labeling of Glu (data set #1)	99%	98%	100%																98%	98%	99%
10	Fractional labeling of Tyr (data set #1)	55%	1%	100%																97%	1%	100%
11	Fractional labeling of Ala (data set #2)				100%	98%	100%													99%	99%	100%
12	Fractional labeling of Gly (data set #2)				97%	96%	100%													98%	97%	100%
13	Fractional labeling of Val (data set #2)				100%	99%	100%													99%	99%	100%
14	Fractional labeling of Leu (data set #2)				99%	99%	100%													99%	99%	100%
15	Fractional labeling of Ile (data set #2)				99%	99%	100%													100%	99%	100%
16	Fractional labeling of Ser (data set #2)				98%	97%	100%													99%	98%	99%
17	Fractional labeling of Phe (data set #2)				100%	99%	100%													100%	99%	100%
18	Fractional labeling of Asp (data set #2)				98%	97%	99%													99%	98%	99%
19	Fractional labeling of Glu (data set #2)				99%	98%	99%													99%	98%	99%
20	Fractional labeling of Tyr (data set #2)				99%	98%	100%													99%	98%	100%
21	Fractional labeling of Ala (data set #3)							95%	94%	100%										97%	96%	98%
22	Fractional labeling of Gly (data set #3)							93%	92%	100%										95%	94%	96%
23	Fractional labeling of Val (data set #3)							97%	96%	100%										98%	97%	99%
24	Fractional labeling of Leu (data set #3)							93%	87%	100%										95%	91%	99%
25	Fractional labeling of Ile (data set #3)							100%	94%	100%										97%	95%	99%
26	Fractional labeling of Ser (data set #3)							96%	94%	100%										97%	96%	99%
27	Fractional labeling of Phe (data set #3)							98%	97%	100%										100%	99%	100%
28	Fractional labeling of Asp (data set #3)							100%	92%	100%										94%	93%	95%
29	Fractional labeling of Glu (data set #3)							97%	91%	99%										95%	93%	97%
30	Fractional labeling of Tyr (data set #3)							98%	97%	100%										100%	99%	100%
31	Fractional labeling of Ala (data set #4)										98%	79%	100%							98%	97%	99%
32	Fractional labeling of Gly (data set #4)										93%	72%	100%							95%	94%	96%
33	Fractional labeling of Val (data set #4)										99%	80%	100%							99%	98%	100%
34	Fractional labeling of Leu (data set #4)										99%	0%	100%							100%	0%	100%
35	Fractional labeling of Ile (data set #4)										98%	71%	100%							97%	95%	100%
36	Fractional labeling of Ser (data set #4)										96%	75%	100%							98%	97%	98%
37	Fractional labeling of Phe (data set #4)										100%	76%	100%							100%	99%	100%
38	Fractional labeling of Asp (data set #4)										96%	70%	100%							96%	95%	98%
39	Fractional labeling of Glu (data set #4)										95%	68%	98%							93%	91%	96%
40	Fractional labeling of Tyr (data set #4)										99%	76%	100%							99%	98%	100%
41	Fractional labeling of Ala (data set #5)													100%	99%	100%				99%	99%	100%
42	Fractional labeling of Gly (data set #5)													97%	96%	100%				99%	98%	100%
43	Fractional labeling of Val (data set #5)													100%	99%	100%				99%	99%	100%
44	Fractional labeling of Leu (data set #5)													99%	99%	100%				99%	99%	100%
45	Fractional labeling of Ile (data set #5)													100%	99%	100%				100%	99%	100%
46	Fractional labeling of Ser (data set #5)													99%	98%	100%				99%	98%	100%
47	Fractional labeling of Phe (data set #5)													100%	99%	100%				100%	99%	100%
48	Fractional labeling of Asp (data set #5)													99%	98%	100%				99%	99%	100%
49	Fractional labeling of Glu (data set #5)													99%	98%	99%				99%	98%	99%
50	Fractional labeling of Tyr (data set #5)													99%	98%	100%				99%	98%	100%
51	Fractional labeling of Ala (data set #6)																99%	98%	100%	100%	99%	100%
52	Fractional labeling of Gly (data set #6)																4%	2%	5%	88%	46%	100%
53	Fractional labeling of Val (data set #6)																99%	99%	100%	100%	99%	100%
54	Fractional labeling of Leu (data set #6)																99%	98%	99%	99%	98%	99%
55	Fractional labeling of Ile (data set #6)																99%	99%	100%	99%	99%	100%
56	Fractional labeling of Ser (data set #6)																97%	96%	97%	97%	96%	98%
57	Fractional labeling of Phe (data set #6)																100%	99%	100%	100%	99%	100%
58	Fractional labeling of Asp (data set #6)																99%	97%	100%	98%	98%	99%
59	Fractional labeling of Glu (data set #6)																99%	98%	99%	99%	98%	99%
60	Fractional labeling of Tyr (data set #6)																100%	0%	100%	100%	0%	100%

Table S6. Goodness-of-fit analysis for ^{13}C-MFA using a network model without the CO_2 exchange reaction v_{71}. Although acceptable fits were obtained for the six individual experiments, combined analysis of all six data sets did not produce a statistically acceptable fit. This suggests that CO_2 exchange reaction v_{71} is required for ^{13}C-MFA.

Fitted data set	No. of fitted measurements*	No. of estimated free fluxes (and G-values)	No. of redundant measurements*	Maximum acceptable SSR at 95% confidence level	SSR (accepted)
[1-^{13}C]glucose	71	16 (8)	47	68	19 (Yes)
[2-^{13}C]glucose	73	16 (10)	47	68	22 (Yes)
[3-^{13}C]glucose	69	16 (10)	43	63	18 (Yes)
[4-^{13}C]glucose	42	14 (0)	28	44	43 (Yes)
[5-^{13}C]glucose	65	14 (10)	41	60	43 (Yes)
[6-^{13}C]glucose	68	14 (8)	46	66	44 (Yes)
COMPLETE-MFA	378	20 (55)	303	353	**467 (No)**

* Number of fitted measurements includes external flux measurements and mass isotopomer measurements that were non-zero. Number of redundant measurements (*n*-*p*) was calculated from on the number of fitted measurements (*n*) minus the number of estimated free fluxes and G-values (*p*).

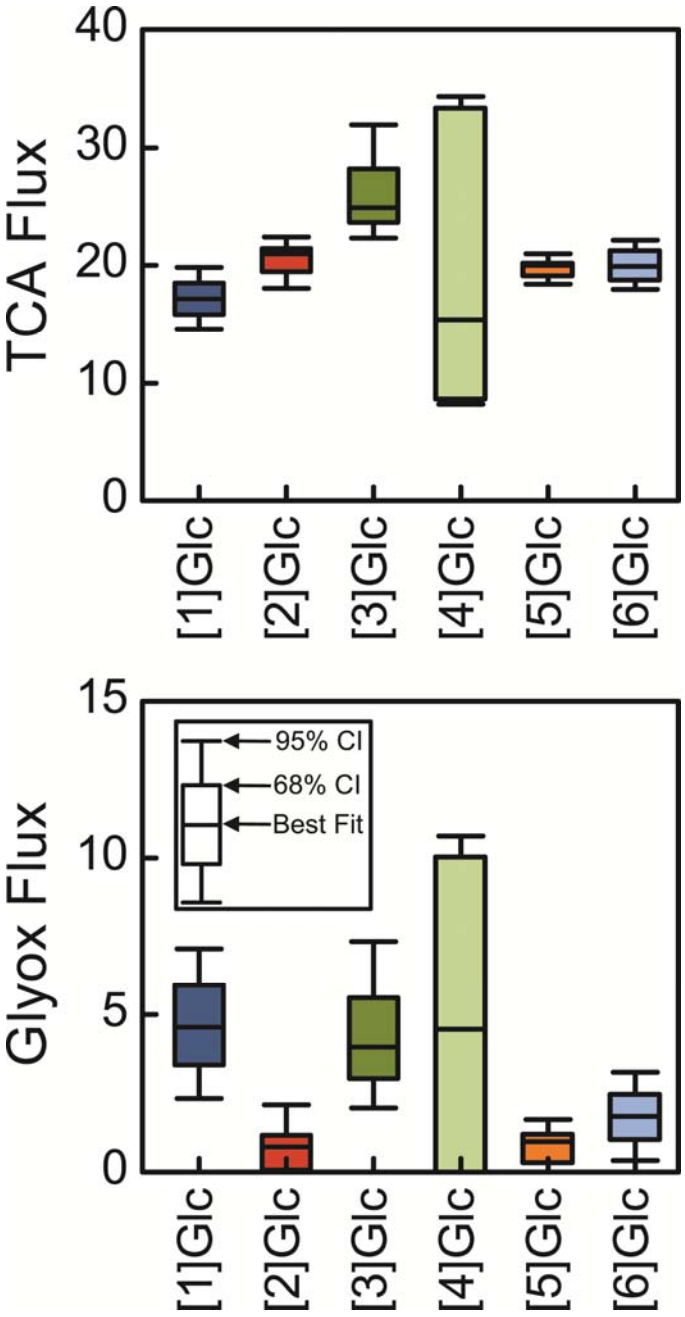

Figure S7. Inconsistent metabolic fluxes were obtained (i.e. the 95% confidence intervals were not overlapping) when fluxes were estimated using an incomplete metabolic network model without the CO_2 exchange reaction (v_{71}). The 68% and 95% confidence intervals are shown for the TCA cycle flux (citrate synthase, v_{21}) and glyoxylate shunt (v_{29}).

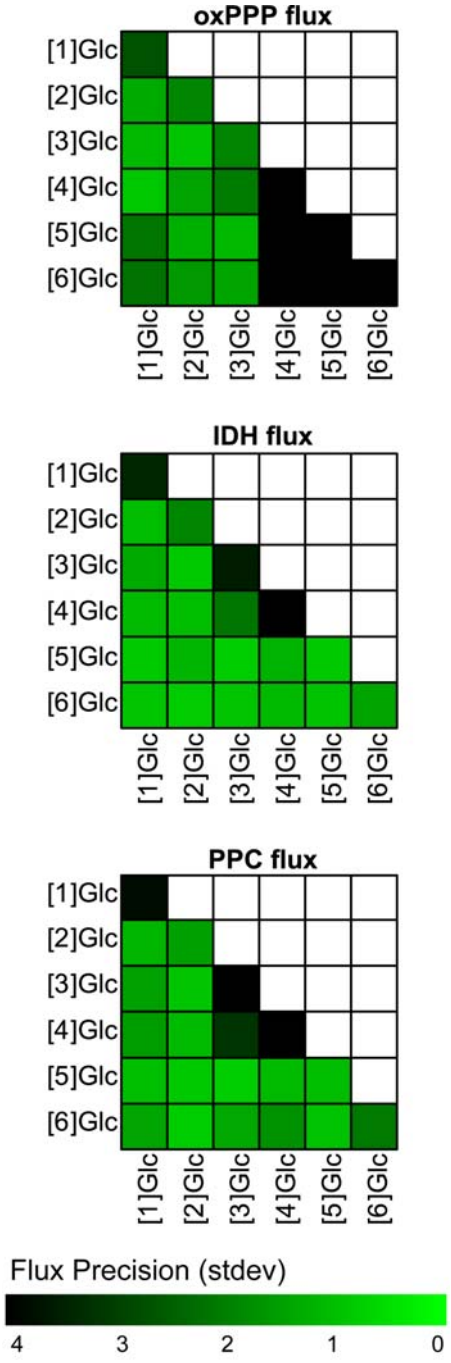

Figure S8. Comparison of the precision of metabolic fluxes estimated using combined analysis of dual ^{13}C-tracer experiments. The flux precisions (stdev) are shown for the oxidative pentose phosphate pathway flux (oxPPP, v_{10}), isocitrate dehydrogenase flux (IDH, v_{23}), and phosphoenolpyruvate carboxylase flux (PPC, v_{33}). Overall, the most precise fluxes were obtained when [2-^{13}C]glucose and [3-^{13}C]glucose tracers were used in parallel.