Supporting Information for

Quantifying Biomass Composition by Gas Chromatography/Mass Spectrometry

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This document contains the following information in support of the primary article:

Example Isotope Ratio Calculation

For all isotope ratio calculations, mass isotopomer distributions (MID) are first corrected for natural isotope abundances using the method of Fernandez et. al.[1] In the example below, we show how the amount of protein-bound aspartate in *E. coli* Δ*pgi* strain is determined using GC-MS data for aspartate fragment at *m/z* 418.

Step 1. Measure the amount of aspartate in fully labeled biomass standard.

First, we measure MIDs for the "fully labeled" biomass standard, and a mixture of the biomass standard with an unlabeled amino acid standard with a known concentration. Here, for the unlabeled amino acid standard, 40 μL of a 2.5 mM (per amino acid) solution was used. Note: the measured biomass Asp content corresponds to the sum of biological Asp and Asn (denoted by Asx). The standard solution contains only Asp. The unlabeled standard is assumed to have natural isotopic labeling (i.e. M0 = 1).

Step 1a. Measure MID for fully labeled biomass standard

Asp418 (M0)	0.0135
Asp418 (M1)	0.0016
Asp418 (M2)	0.0127
Asp418 (M3)	0.1639
Asp418 (M4)	0.8126

"Labeled" $L = M(N) + M(N-1) = 0.1639 + 0.8126 = 0.9765$ **Eq. 1a**

"Unlabeled" $U = M(0) = 0.0135$ **Eq. 1b**

Notes: The M0 (~1.4%) is coming from the unlabeled inoculum. The M3 (~16%) is a result of incomplete labeling of [U-^{13}C]glucose tracer (~98.5% ^{13}C) as well as fixation of unlabeled atmospheric CO_2.

Next, we calculate the fractions of L and U:

$$X_{L,BM} = \frac{L}{L+U} = 0.986$$

$$X_{U,BM} = 1 - X_{L,BM} = 0.014$$

Step 1b. Measure MIDs for mixtures of fully labeled biomass + 100 nmol unlabeled Asp (3 replicates)

	Rep 1	*Rep2*	*Rep 3*
Asp418 (M0)	0.4427	0.4323	0.4134
Asp418 (M1)	0.0000	0.0000	0.0000
Asp418 (M2)	0.0069	0.0068	0.0072
Asp418 (M3)	0.0927	0.0945	0.0964
Asp418 (M4)	0.4619	0.4699	0.4848
"L_{mix}"	**0.5546**	**0.5644**	**0.5812**
"U_{mix}"	**0.4427**	**0.4323**	**0.4134**

These values are corrected for the unlabeled content of the labeled biomass standard as follows:

$$\left(\frac{L}{U}\right)_{corrected} = \frac{L_{mix}}{U_{mix} \times X_{L,BM} - L_{mix} \times X_{U,BM}} \qquad \textbf{Eq. 2}$$

	Rep 1	*Rep 2*	*Rep 3*	*Avg*
L/U (corr.)	1.292	1.348	1.454	1.365

Amount of Asp in the unlabeled amino acid standard added:

40µL x 2.5 mM = 100 nmol

Thus, the amount of Asp in fully labeled biomass is:

1.365 x 100 nmol = 136.5 nmol

Step 2. Measure the amount of aspartate in E. coli Δpgi strain.

Since *E. coli* Δ*pgi* cultures were grown on naturally labeled glucose, they are assumed to have only naturally labeling (i.e. M0 = 1, after correction for natural abundances).

Step 2a. Measure MIDs for mixtures of fully labeled biomass + Δ*pgi* samples
(4 replicates)

	Rep 1	*Rep 2*	*Rep 3*	*Rep 4*
Asp418 (M0)	0.5182	0.5086	0.4836	0.4978
Asp418 (M1)	0.0000	0.0000	0.0000	0.0000
Asp418 (M2)	0.0059	0.0058	0.0064	0.0064
Asp418 (M3)	0.0807	0.0817	0.0856	0.084
Asp418 (M4)	0.3997	0.4076	0.4275	0.416
"$L_{pgi,mix}$"	**0.4804**	**0.4893**	**0.5131**	**0.5000**
"$U_{pgi,mix}$"	**0.5182**	**0.5086**	**0.4836**	**0.4978**

These values are corrected in the same way as above for the presence of unlabeled material in the "labeled" biomass standard.

Then, the Asp content is calculated by multiplying the corrected L/U by the known Asp content of the labeled biomass standard (i.e. 136.5 nmol)

	Rep 1	*Rep 2*	*Rep 3*	*Rep 4*
L/U (corr.)	0.952	0.989	1.092	1.033
Asp (nmol)	143.3	138.1	125.0	131.4

Thus, the amount of Asp in *E. coli* Δpgi strain is: 134.6 ± 7.9 nmol

We wish to report the composition in μmol/g_{dw}, so we must also have the dry weight of the sample. This was measured independently for the Δ*pgi* strain to be 0.306 mg_{dw}/(mL*OD). All biomass samples here were an equivalent of 1 mL of OD = 1.

$$134.6\ \frac{nmol\ Asp}{mL-OD} \times \frac{1}{0.306\ \frac{mg_{dw}}{mL-OD}} = 439\ \frac{nmol}{mg_{dw}} = 439 \frac{\mu mol}{g_{dw}}$$

The standard error of the mean is calculated as:

$$\sigma = 7.9\ \frac{nmol}{mL - OD} \times \frac{1}{0.306 \frac{mg_{dw}}{mL - OD}} = 25.7\ \frac{\mu mol}{g_{dw}}$$

$$SEM = \frac{\sigma}{\sqrt{n}} = \frac{25.7}{\sqrt{4}} = 12.8\ \frac{\mu mol}{g_{dw}}$$

Note: In the primary article, the reported Asx values are an average of three values, based on the analysis of Asp fragments Asp302, Asp390, and Asp418, each calculated as above.

Validation of RNA and Glycogen Measurements: The Labeling Switch Experiment.

The RNA and glycogen measurements were validated to ensure that the target analytes were the desired biomass components, and not other intracellular metabolites such as sugar phosphates. A culture of WT *E. coli* was grown to an OD of 0.5 on unlabeled glucose, was centrifuged and then re-suspended in media containing only fully [U-^{13}C] labeled glucose (at t = 0). Figure S1 shows the culture data following that point, with respect to measured OD_{600} as well as the calculated percentage of new biomass (i.e. generated after t=0). Figure S2 shows the measured RNA and glycogen labeling over the time course. Rapid and complete labeling would have been indicative of the measurement of fast-turnover species such as intracellular metabolites, while a labeling trend similar to the increasing fraction of new labeled biomass would be indicative of the measurement of slow-turnover biomass components. The latter was observed, validating the RNA and glycogen measurements.

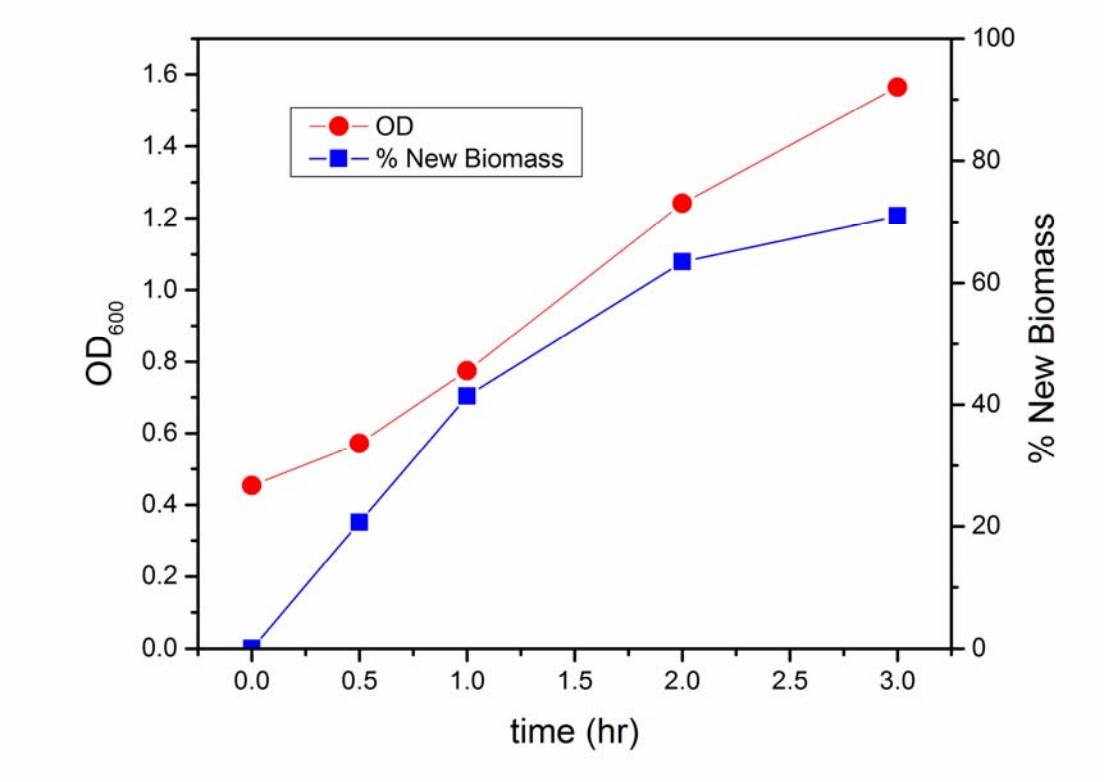

Figure S-1. Culture data from the labeling switch experiment.

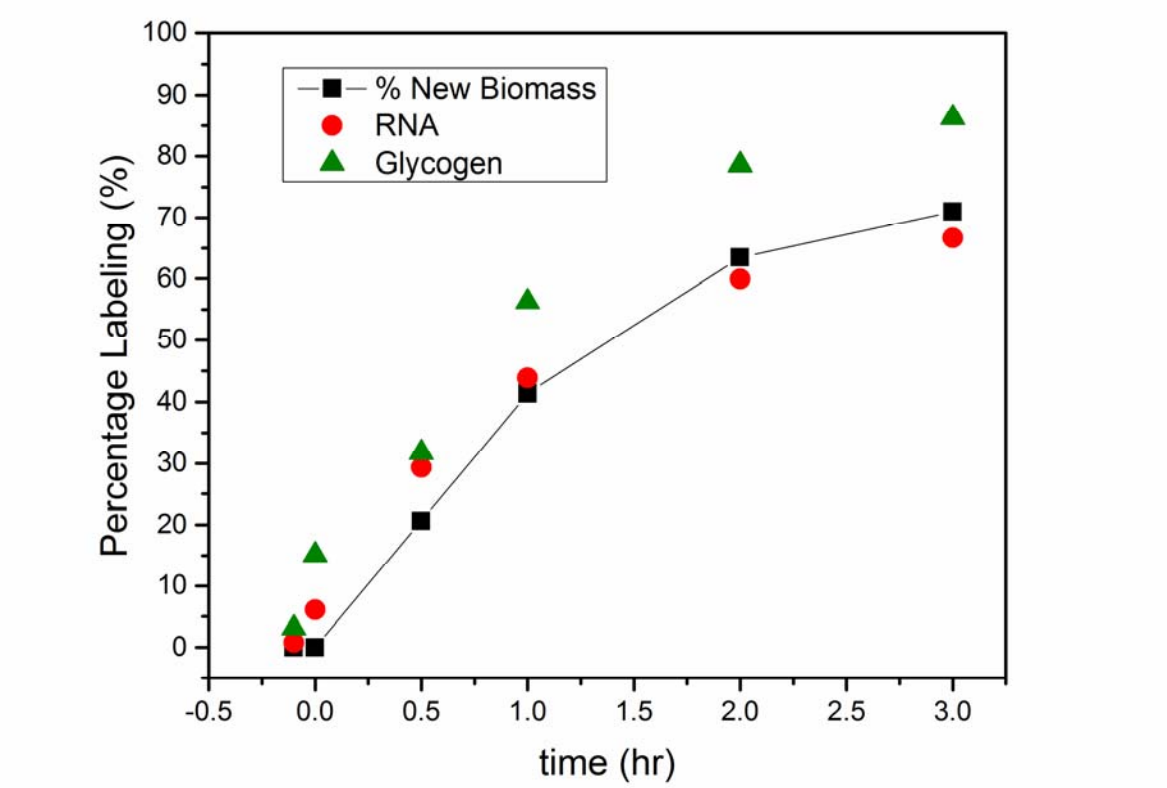

Figure S-2. Observed labeling of RNA and glycogen over time after the switch to [U-^{13}C] labeled substrate. The measurement before t=0 was prior to the switch. Overall, the trends indicate the measurement of slow-turnover biomass components.

Validation of Method Consistency: Analysis of Labeled and Unlabeled Biomass.

Two *E. coli* cultures were grown in parallel, one on unlabeled glucose and one on fully [U-^{13}C] labeled glucose. The labeled biomass was analyzed using unlabeled chemical standards, while the unlabeled biomass was analyzed using a reference fully labeled biomass reference. This labeled biomass standard was one of a large stock of fully labeled *E. coli* culture, which had been previously grown and characterized relative to unlabeled chemical standards. The consistency of results from both approaches demonstrates the reproducibility and flexibility of these methods. Results for the measured biomass components are shown below in Figures S3-S5.

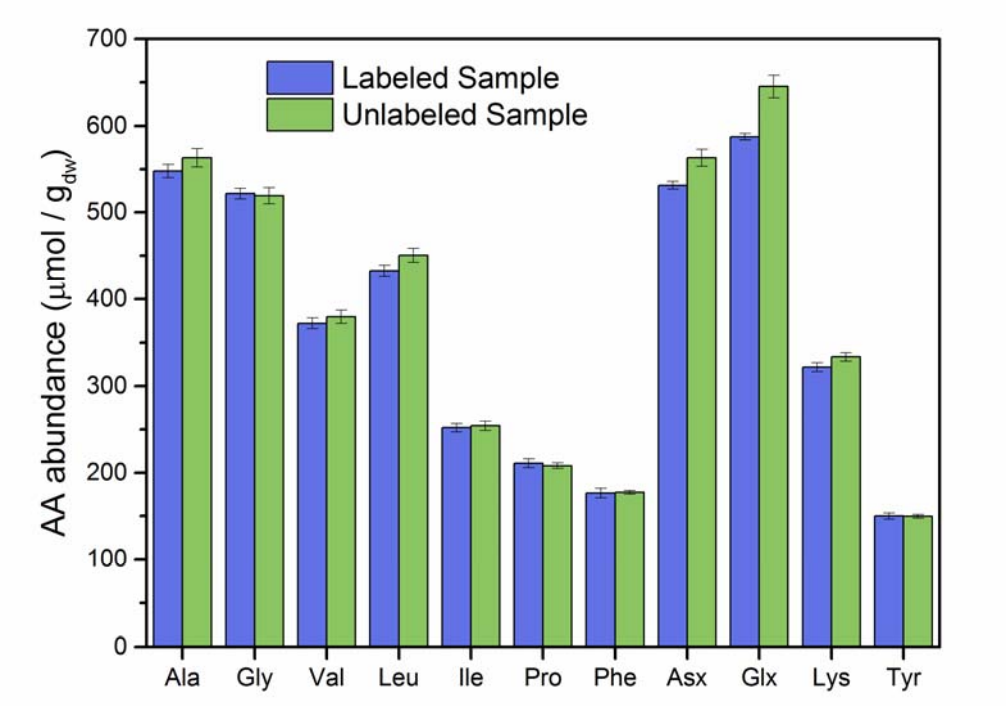

Figure S-3. The abundance of amino acids in *E. coli* biomass as measured by both approaches. Error bars indicate typical measurement uncertainty for both approaches.

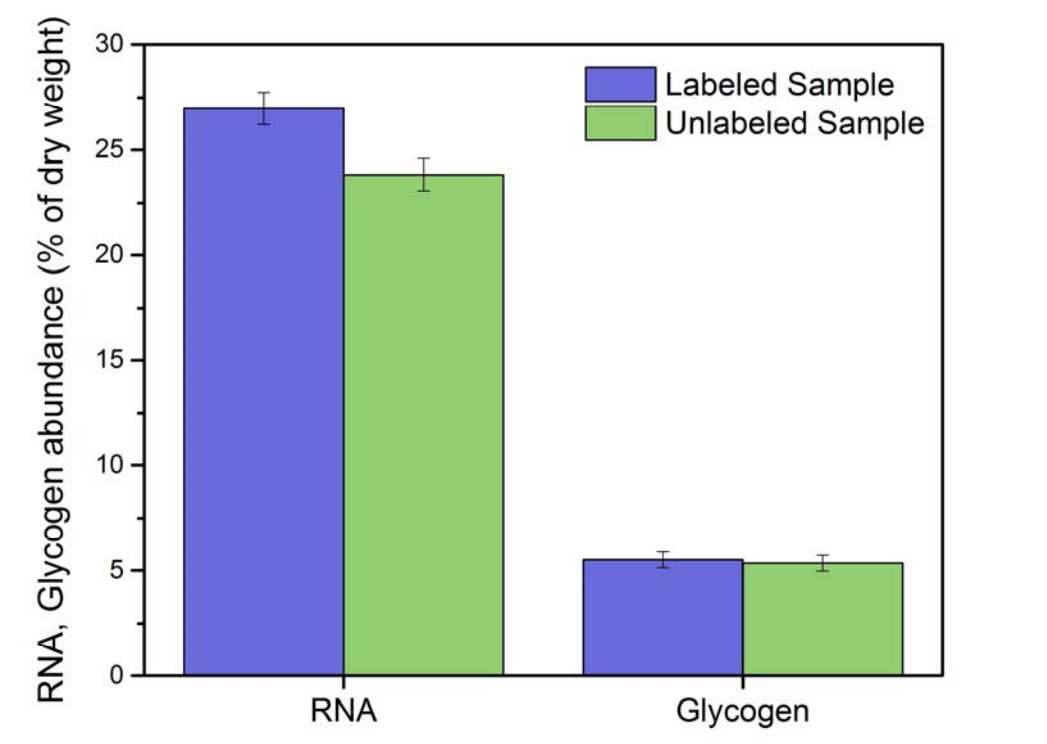

Figure S-4. The abundance of RNA and glycogen in *E. coli* biomass as measured by both approaches. Error bars indicate typical measurement uncertainty.

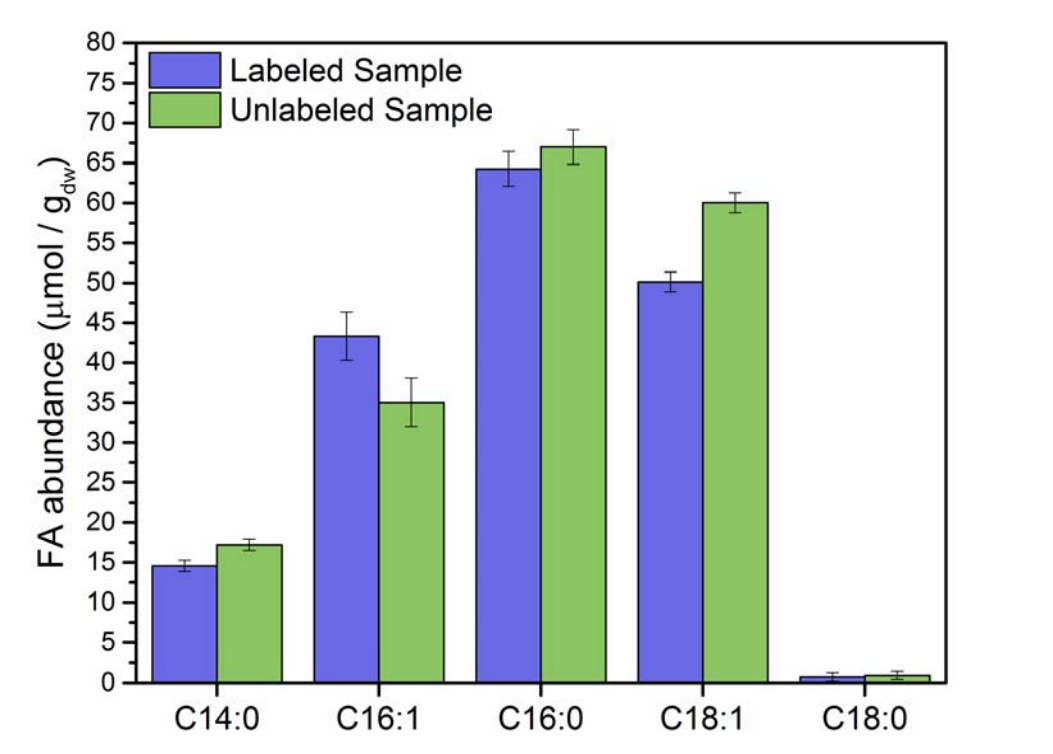

Figure S-5. The abundance of fatty acids in *E. coli* biomass as measured by both approaches. Error bars indicate typical measurement uncertainty for both approaches.

References

(1) Fernandez, C. a; Des Rosiers, C.; Previs, S. F.; David, F.; Brunengraber, H. *J. Mass Spectrom.* **1996**, *31*, 255–262.