# Analysis of Stable Isotope Assisted Metabolomics Data Acquired by High Resolution Mass Spectrometry

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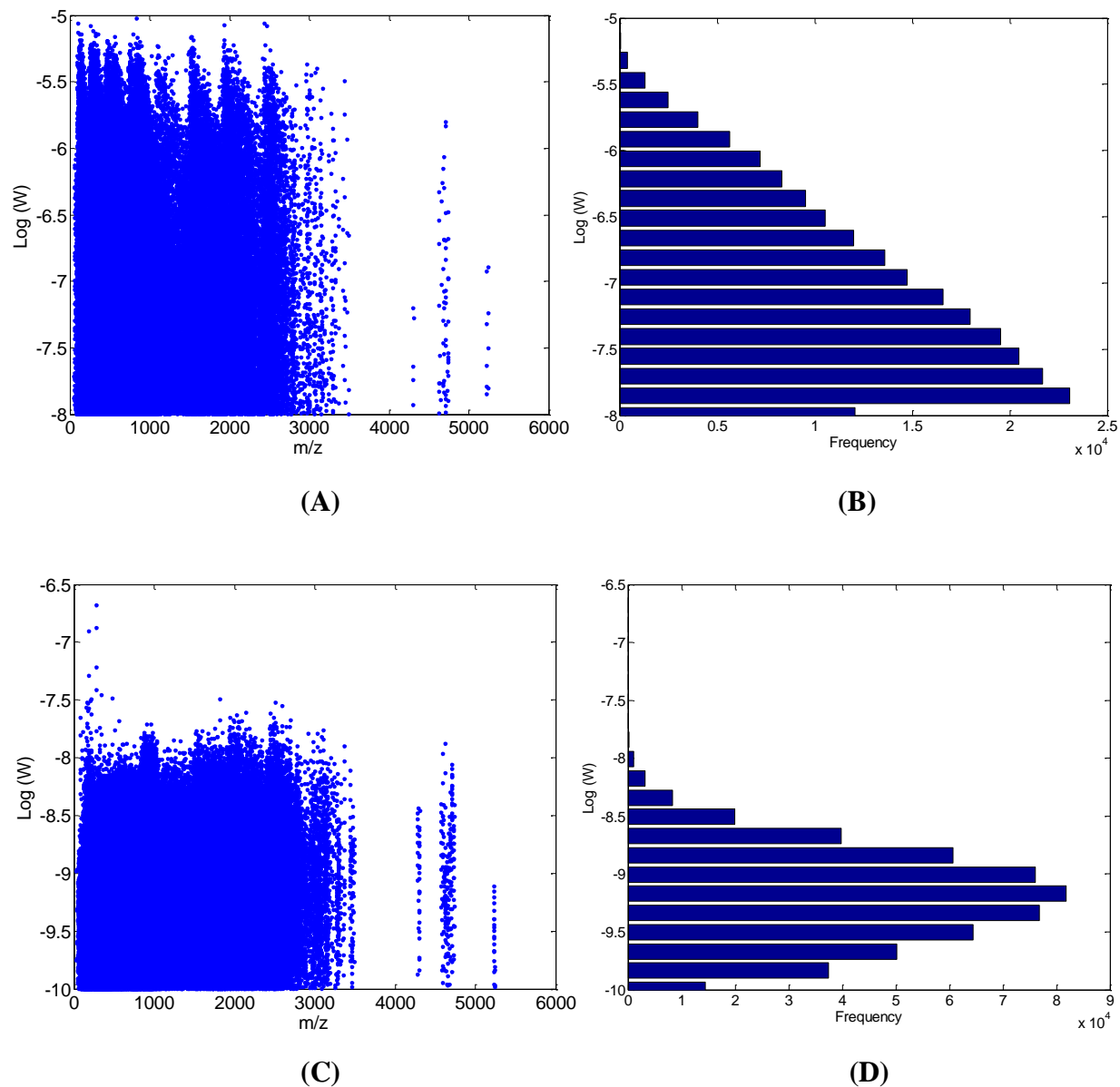
**Fig. S1** Relationship of simulated maximum weight factor and the m/z values of metabolite. (A) is the distribution of simulated maximum weight factors for metabolites labeled by tracer atom $^{13}C$ and 7% of additive noise on the abundance of isotopic peaks. (B) shows the histogram distribution of the values of log(w) in (A). (C) is the distribution of simulated maximum weight factors for metabolites labeled by tracer atom $^{13}C$ and 7% of multiplicative noise on the abundance of isotopic peaks. The Pearson's correlation coefficient between the simulated weight factors and m/z values in (A) is 0.0095 with a p-value of $8.3 \times 10^{-6}$, while the correlation is 0.1752 with a p-value 0 of in (C). (D) shows the histogram distribution of the values of log(w) in (C).
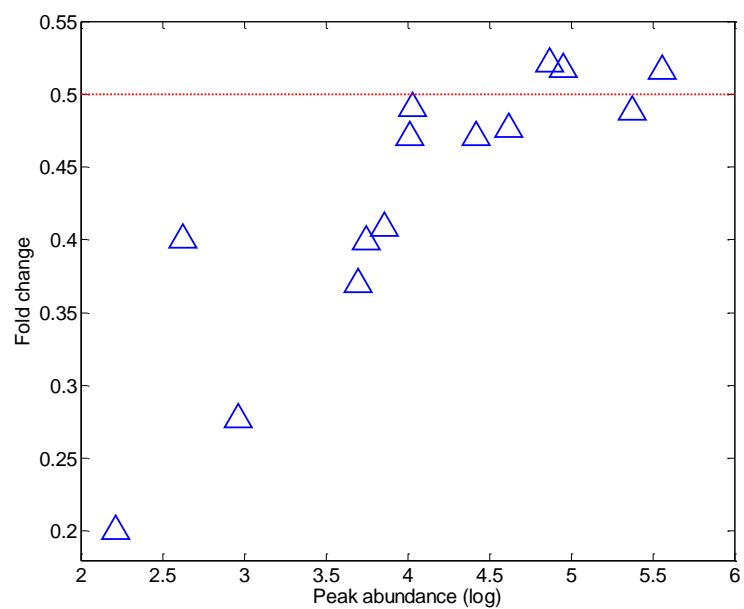
**Fig. S2** The correlation between the peak abundance and fold change. The Pearson's correlation coefficient between the fold change and peak abundance is 0.8615 with a p-value of $7.5 \times 10^{-5}$.
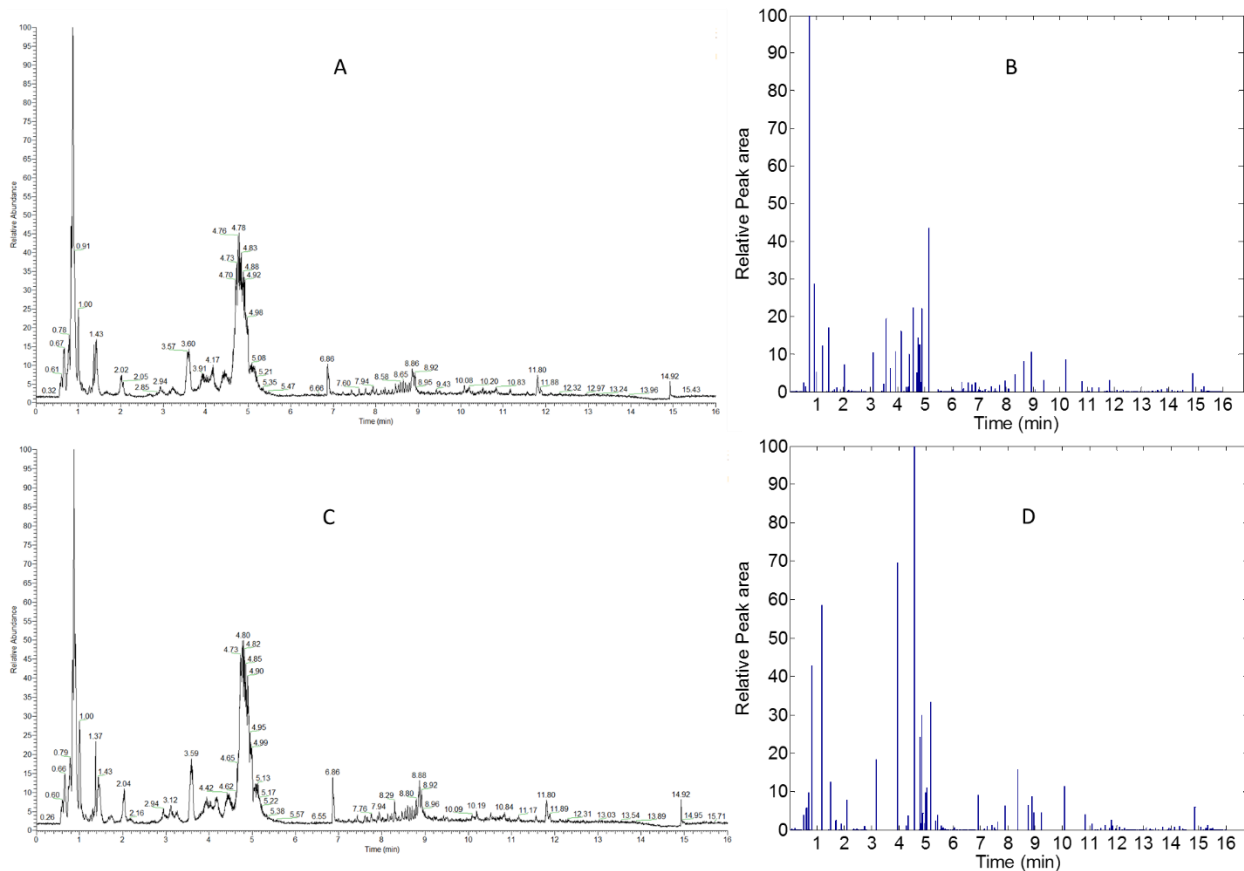
**Fig. S3** Two sample TICs. (A) is the TIC of one sample which was randomly selected from the unlabeled samples. (B) is the reconstructed TIC of the same sample after spectrum deconvolution. (C) is the TIC of one sample was randomly selected from the labeled samples. (D) is the reconstructed TIC of the labeled sample after spectrum deconvolution.
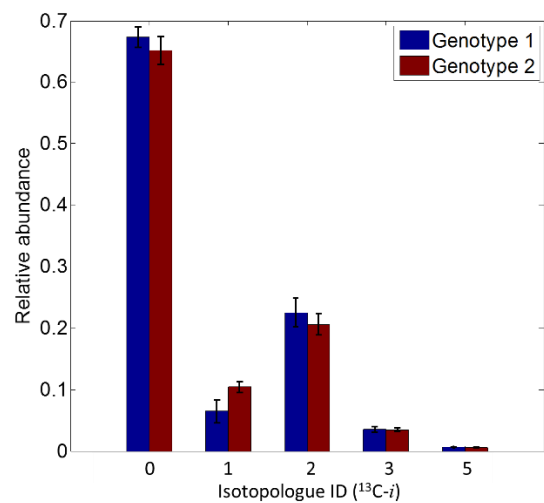
**Fig. S4** Isotopologues of ʟ-glutathione (reduced), the retention time is 4.75 min, which is confirmed with our standard.

**Table S1.** Effects of noise type, level of noise, and trace atoms on the magnitude of weight factor. Each simulation was performed three times and the standard deviation of the maximum simulated weight factors under each set of conditions is calculated.

| | 2% noise | | 5% noise | | 7% noise | | 10% noise | |
|---|---|---|---|---|---|---|---|---|
| | additive | multiplicative | additive | multiplicative | additive | multiplicative | additive | multiplicative |
| $^{2}H$ | $2.6 \times 10^{-14} \pm 0$ | $3.2 \times 10^{-11} \pm 0$ | $2.6 \times 10^{-14} \pm 0$ | $3.2 \times 10^{-11} \pm 0$ | $8.8 \times 10^{-6} \pm 6.0 \times 10^{-7}$ | $3.8 \times 10^{-7} \pm 7.3 \times 10^{-8}$ | $0.59 \pm 0.06$ | $0.06 \pm 0.02$ |
| $^{13}C$ | $9.7 \times 10^{-14} \pm 0$ | $2.5 \times 10^{-11} \pm 0$ | $9.7 \times 10^{-14} \pm 0$ | $2.5 \times 10^{-11} \pm 0$ | $8.9 \times 10^{-6} \pm 6.9 \times 10^{-7}$ | $2.7 \times 10^{-7} \pm 1.9 \times 10^{-7}$ | $0.59 \pm 0.009$ | $0.03 \pm 0.02$ |
| $^{15}N$ | $1.4 \times 10^{-12} \pm 0$ | $2.0 \times 10^{-11} \pm 0$ | $1.4 \times 10^{-12} \pm 0$ | $2.0 \times 10^{-11} \pm 0$ | $5.8 \times 10^{-6} \pm 3.4 \times 10^{-7}$ | $3.4 \times 10^{-8} \pm 2.6 \times 10^{-9}$ | $0.47 \pm 0.04$ | $4.4 \times 10^{-3} \pm 5.2 \times 10^{-4}$ |
| $^{18}O$ | $1.4 \times 10^{-11} \pm 0$ | $3.4 \times 10^{-11} \pm 0$ | $1.4 \times 10^{-11} \pm 0$ | $3.4 \times 10^{-11} \pm 0$ | $8.1 \times 10^{-6} \pm 4.8 \times 10^{-7}$ | $2.6 \times 10^{-7} \pm 1.2 \times 10^{-8}$ | $0.59 \pm 0.05$ | $0.04 \pm 0.02$ |
| $^{34}S$ | $4.7 \times 10^{-15} \pm 0$ | $2.0 \times 10^{-11} \pm 0$ | $4.7 \times 10^{-15} \pm 0$ | $2.0 \times 10^{-11} \pm 0$ | $2.4 \times 10^{-6} \pm 1.9 \times 10^{-6}$ | $7.7 \times 10^{-9} \pm 1.4 \times 10^{-9}$ | $0.20 \pm 0.05$ | $1.2 \times 10^{-3} \pm 3.6 \times 10^{-4}$ |
| $^{13}C\text{-}^{15}N$ | $3.8 \times 10^{-13} \pm 0$ | $2.5 \times 10^{-11} \pm 0$ | $3.8 \times 10^{-13} \pm 0$ | $2.5 \times 10^{-11} \pm 0$ | $8.5 \times 10^{-6} \pm 6.2 \times 10^{-7}$ | $1.9 \times 10^{-7} \pm 4.4 \times 10^{-8}$ | $0.57 \pm 0.04$ | $0.03 \pm 0.004$ |

**Table S2.** Metabolite quantification by analysis of SIAM data acquired from two sample groups containing mixtures of $^{13}$C-labled amino acids.

| Amino acids | Labeling status | [a]Fold change | p-value |
|---|---|---|---|
| L-alanine | $^{13}$C$_3$-labeled | 0.40 | $1.2 \times 10^{-2}$ |
| L-aspartic acid | $^{13}$C$_4$-labeled | 0.41 | $4.4 \times 10^{-2}$ |
| L-arginine | $^{13}$C$_6$-labeled | 0.48 | $7.4 \times 10^{-3}$ |
| L-glutamic acid | $^{13}$C$_5$-labeled | 0.52 | $4.9 \times 10^{-3}$ |
| L-glycine | $^{13}$C$_2$-labeled | 0.40 | $5.1 \times 10^{-2}$ |
| L-histidine | $^{13}$C$_6$-labeled | 0.37 | $4.0 \times 10^{-2}$ |
| L-isoleucine/ L-leucine | $^{13}$C$_6$-labeled | 0.52 | $1.0 \times 10^{-2}$ |
| L-lysine | $^{13}$C$_6$-labeled | 0.47 | $2.5 \times 10^{-3}$ |
| L-methionine | $^{13}$C$_5$-labeled | 0.47 | $3.2 \times 10^{-2}$ |
| L-phenylalanine | $^{13}$C$_9$-labeled | 0.49 | $2.5 \times 10^{-2}$ |
| L-proline | $^{13}$C$_5$-labeled | 0.28 | $3.7 \times 10^{-2}$ |
| L-threonine | $^{13}$C$_4$-labeled | 0.20 | $3.5 \times 10^{-2}$ |
| L-tyrosine | $^{13}$C$_9$-labeled | 0.52 | $1.5 \times 10^{-2}$ |
| L-valine | $^{13}$C$_5$-labeled | 0.49 | $1.7 \times 10^{-2}$ |

[a] fold change for 0.5:1 to 1:1.

**Table S3.** Percent abundance distribution of $^{13}C$ isotopologues of 9 nucleotide/nucleotide sugar metabolites before and after applying the iterative linear regression (ILR) algorithm. Data shown for cells cultured in 5 mM [U-$^{13}C$]-glucose for 18 hr.

| Isotopologue | Abundance Distribution (Percent Enrichment) | | | | | | | | | Experimental/ILR |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | ATP | CTP | GTP | GSSG | UDP | UTP | UDP-GLC | UDP-GLCUR | UDP-GNAc | |
| 0 | 19.2 | 72.0 | 22.9 | 48.5 | 40.2 | 38.2 | 1.5 | 2.4 | 4.6 | EXP |
| | 19.4 | 73.0 | 23.1 | 53.1 | 42.5 | 40.9 | 1.5 | 2.6 | 5.1 | ILR |
| 1 | 1.7 | 0 | 0 | 5.9 | 3.7 | 3.1 | 0 | 0 | 0.8 | EXP |
| | 1.8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ILR |
| 2 | 5.9 | 0.9 | 6.1 | 38.8 | 5.6 | 0 | 0 | 0.1 | 2.0 | EXP |
| | 5.8 | 0.9 | 6.1 | 42.5 | 5.9 | 0 | 0 | 0.1 | 2.3 | ILR |
| 3 | 5.4 | 0.6 | 5.7 | 2.7 | 4.8 | 0 | 0.4 | 1.9 | 2.0 | EXP |
| | 4.8 | 0.6 | 5.1 | 0 | 4.5 | 0 | 0.4 | 2.0 | 1.8 | ILR |
| 4 | 3.8 | 0 | 4.5 | 3.2 | 4.8 | 4.2 | 0 | 0.2 | 1.3 | EXP |
| | 3.9 | 0 | 4.5 | 3.5 | 4.7 | 4.5 | 0 | 0 | 1.2 | ILR |
| 5 | 59.2 | 20.8 | 55.6 | 0 | 0 | 40.7 | 3.8 | 5.6 | 5.8 | EXP |
| | 59.5 | 21.1 | 56.0 | 0 | 0 | 43.3 | 4.0 | 6.0 | 6.2 | ILR |
| 6 | 3.8 | 4.0 | 4.3 | 0.4 | 0 | 7.0 | 38.3 | 28.8 | 19.8 | EXP |
| | 3.9 | 2.8 | 4.3 | 0.4 | 0 | 5.0 | 39.9 | 30.1 | 21.0 | ILR |
| 7 | 0.7 | 1.7 | 0.8 | 0 | 23.9 | 6.6 | 1.6 | 4.6 | 5.6 | EXP |
| | 0.7 | 1.7 | 0.8 | 0 | 25.3 | 6.1 | 0 | 1.5 | 3.3 | ILR |
| 8 | 0.2 | 0 | 0.1 | 0.4 | 16.9 | 0 | 4.7 | 6.3 | 16.9 | EXP |
| | 0.2 | 0 | 0.1 | 0.5 | 17.0 | 0 | 5.0 | 6.6 | 18.3 | ILR |
| 9 | | | | | | 0.1 | 3.1 | 0 | 4.4 | EXP |
| | | | | | | 0 | 2.9 | 0 | 2.7 | ILR |
| 10 | | | | | | | 4.1 | 6.1 | 0 | EXP |
| | | | | | | | 4.1 | 6.5 | 0 | ILR |
| 11 | | | | | | | 37.5 | 31.6 | 14.9 | EXP |
| | | | | | | | 39.3 | 33.5 | 16.6 | ILR |
| 12 | | | | | | | 3.8 | 6.6 | 5.2 | EXP |
| | | | | | | | 1.6 | 5.1 | 4.3 | ILR |
| 13 | | | | | | | 1.3 | 5.4 | 12.2 | EXP |
| | | | | | | | 1.3 | 5.6 | 13.2 | ILR |
| 14 | | | | | | | | 0.4 | 2.8 | EXP |
| | | | | | | | | 0.4 | 2.3 | ILR |
| 15 | | | | | | | | | 1.6 | EXP |
| | | | | | | | | | 1.7 | ILR |
| 16 | | | | | | | | | 0.1 | EXP |
| | | | | | | | | | 0.2 | ILR |